Int J Clin Exp Pathol 2018;11(9):4461-4471
www.ijcep.com /ISSN:1936-2625/IJCEP0077975

Original Article

Tanshinone IIA protects against polyethylene particle-induced osteolysis response in a mouse calvarial model

Jun Yao[1,3*], Shiting Ma[1,3*], Wenyu Feng[1,3], Yan Wei[2,3], Huiping Lu[2,3], Gang Zhong[1,3], Zhengyuan Wu[1,3], Hongtao Wang[1,3], Wei Su[1,3*], Jia Li[2,3*]

Departments of [1]Orthopaedic, [2]Pathology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, P. R. China; [3]Research Centre for Regenerative Medicine and Guangxi Key Laboratory of Regenerative Medicine, Guangxi Medical University, Nanning, Guangxi, P. R. China. [*]Equal contributors.

Received April 16, 2018; Accepted July 31, 2018; Epub September 1, 2018; Published September 15, 2018

Abstract: The expression of β-catenin in detectable aseptic loosening after joint replacement and the surrounding osteolysis of the prosthesis is primarily caused by the abrasive particles introduced by the prosthesis, which results in a shortened service life of the prosthesis. Recent studies have shown that debris can induce many cytokines associated with osteolysis. In particular, RANKL directly stimulates osteoclast formation and activity. Thus, we hypothesize that the osteolysis induced by wear particles can be prevented by inhibiting the RANKL signaling pathway. In this study, we established a C57BL/J6 mouse calvarial model of PE granule induced osteolysis, and studied the inhibitory action of tanshinone IIA on osteoclast formation. Eight-week-old male c57BL/J6 mouse were randomly divided into four groups: Sham group (no PE particle-induced + PBS), positive group (PE particle-inducted + PBS), low dose group (PE particle-induced + 1 ug/g tanshinone IIA), and high-dose group (PE granule-induced + 2 ug/g tanshinone II). After 21 days, the mice were executed and the calvaria were collected and processed for micro-CT scan and histomorphometry analysis. Compared to the positive subgroup, Tanshinone IIA significantly reduced bone absorption induced by PE granules and inhibited the formation and activity of osteoclasts. In addition, ELISA test showed that tanshinone IIA significantly reduced OSCAR and CTX-1 expression. Further, tanshinone IIA enhanced the formation of OPG, thus reducing osteoclast damage to the bone around the implant. Overall, these data indicate that tanshinone IIA represents a promising drug for the treatment of bone absorption by particles and can be a new method of treatment for prophylaxis of aseptic loosening.

Keywords: Aseptic loosening, tanshinone IIA, RANKL, osteoclast, osteolysis

Introduction

Currently, total joint arthroplasty (TJA) is the most effective approach for treating patients with severe joint disease. There are about 700,000 cases of TJA in the United States each year, and it is expected to reach four million by 2030 [1]. However, the dissolution of the bone around the prosthesis and subsequent aseptic loosening remains the main reason for surgical failure, and there is no ideal treatment for this complication [2-6]. Joint revision surgery is the most frequent way to treat aseptic looseness, but this approach does not guarantee the stability of the prosthesis or preclude recurrence of this complication. Some studies have shown that bone absorption is the same pathological process as osteoporosis and aseptic loosening. This process arises mainly due to the presence of titanium alloy, bone cement and polyethylene wear particles, which induce the activation of macrophages. Subsequently, these cells secrete a large number of inflammatory cytokines, thereby activating RANKL signaling to stimulate osteoclast formation, which eventually dissolves the bone around the prosthesis. Particularly, activation of the RANK-RANKL-OPG signaling axis is critical in the pathogenesis of prosthesis-associated osteolysis. Since the research community has recognized that the aseptic loosening of prosthesis was a result of particle-induced wear, our group and

others have been actively seeking a way to prevent particle-induced wear to overcome the process of osteolysis [6].

Aseptic loosening of artificial joints is facilitated by mechanical and biotic causes and results in biological inflammatory processes [4, 6]. Many studies have taken into account the release of wear particles around the prosthesis, which suggests that osteolysis is a critical element in the aseptic looseness [7-11]. Indeed, Carl and colleagues confirmed that PE particles can induce osteolysis around artificial joints [12]. The chronic inflammatory response caused by abrasive particles leads to the accumulation and activation of macrophages, fibroblasts, lymphocytes, and osteoclast cells. These cells secrete a large number of cytokines including interleukin-6 (IL-6), interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), prostaglandin E2 (PGE-2), which aggravate the inflammatory response [13]. These cytokines, especially those produced by macrophages, can also activate RANKL [14]. Crotti [15] and Kim [16] found that the looseness of prosthesis was not only related to an abundance of inflammatory cells and their resulting inflammation factors, but also to the expression of RANKL, RANK and OPG. Ren and his colleague [17] injected the PE particles into the calvarial surface of the RANK gene knockout mouse, which proved that the RANKL gene expression in mice increased, and the bone absorption of osteoclast induced by PE particles was observed in RANK+/+ but not RANKL-/- mice. RANK is the only receptor for RANKL. OPG and RANKL can be used as markers of osteolysis around prostheses [18]. RANKL and its receptor RANK and OPG are critical in the regulation of osteoclast formation. RANKL combines with RANK to promote osteoclast differentiation and maturation by preventing osteoclast apoptosis. This is a key factor that results in osteolysis around the prosthesis. Osteoblasts and BMSC secrete OPG, and OPG combined with RANKL and RANK forms a complex, preventing RANKL from inducing osteoclast differentiation and maturity and promoting bone rebuilding [19, 20]. Consequently, the balance between OPG and RANKL is crucial for the prevention of aseptic loosening [21].

Tanshinone IIA is an active ingredient extracted from rhizome of salvia miltiorrhiza root, which is reported to have a positive effect on osteoporosis [22, 23]. Kim et al. found that a low concentration of tanshinone IIA (1 ug/mL) completely inhibited osteoclast formation [24]. Also, tanshinone can decrease TRAP-positive multinucleated osteoclast formation and inhibit osteoclast formation by inhibiting the expression of c-fos and NFATc1 induced by RANKL [25]. Our group found that a concentration of tanshinone IIA of 2-4 ug/ml was effective in inhibiting the osteoclast precursor cells. We also found that tanshinone IIA could inhibit the destruction of the calvaria *in vivo* in a dose-dependent fashion. We hypothesized that tanshinone IIA can inhibit RANKL-induced osteoclastogenesis and bone absorption, which suggested that tanshinone IIA has great potential for the treatment of osteolysis. Nevertheless, these data were only at the cell level in vitro and have not been applied to animal models. Consequently, we established a mouse model for PE particle-induced osteolysis of calvaria to observe the extent of calvarial damage and to detect specific cytokines associated with osteoclasts and osteoblasts. We found that tanshinone IIA can inhibit the formation of osteoclasts, and we explored the potential effects of tanshinone IIA against osteolysis. Finally, we apply tanshinone IIA to clinical prophylaxis and treatment of bone absorbing disease.

Materials and methods

Preparation of PE particle

UHMWPE was obtained from Clariant (Gersthofer, Germany) [26]. The average diameter of PE particles is 1.84 ± 1.50 μm (range 0.14-12.1 um), and 90% of which are smaller than 1 um. To remove the attached toxin, we soaked PE particles in 75% ethanol solution for 48 h, then the standard ethylene oxide (ETO) was used to disinfect [27, 28], and finally we used phosphate buffered saline (PBS) to wash three times and the final concentration of 100 mg/ml is prepared with PBS.

Reagents and mouse experiments

Tanshinone IIA was obtained from Chengdu Munster Biotechnology (Sichuan, China). Eight-week old male C57BL/J6 mice were provided by the laboratory animal center of Guangxi Medical University and split into four groups at random (six for each group): Sham group (no PE particle-induced + PBS); a Positive group (PE particle-inducted + PBS); a Low dose group (PE particle-induced + 1 ug/g tanshinone IIA); and

a high-dose group (PE granule-induced + 2 ug/g tanshinone II). The study was done in regenerative medicine and pathology laboratories. The ethics review was approved by the animal ethics committee of Guangxi Medical University in accordance with principles and procedures [No. 201702001].

Model establishment

The model of calvarial osteolysis induced by PE particle was based on previous research [26, 29]. Using 2% pentobarbital sodium (0.01 ml/g) for anesthesia, 1 cm × 1 cm skull skin was exposed, then we scraped the skull periosteum and sutured the skin closed. The periosteum of the sham group was injected with 100 ul PBS. The other three groups were injected with PE particles (100 ul, 100 mg/ml). The drug was administered every other day. The third day after surgery, the sham group and the vehicle group were injected with 100 ul PBS, and the other two groups were respectively injected with tanshinone IIA 1 ug/g, 2 ug/g. After 21 days, mice were anesthetized and executed by cervical dislocation, and blood was collected from the femoral artery for analysis by ELISA. Crania were planed for mCT examination and pathological analysis (TRAP and HE staining).

Micro-computed tomography (micro-CT) scanning

In order to remove PE particles, calvaria were mixed in formalin 24 hours before scanning. Samples were scanned by a HR micro-CT system (Skyscan 1076; Skyscan, Kontich, Belgium) using the following parameters: 18 mm isometric resolution, X-ray energy was set to 80 kv and 100 mA, to 0.9° Angle rotate 180° [30]. The three-dimensional (3D) images of the mice calvaria in each group were exported to computer for analysis. In order to study the 3D images of each calvaria, a region with PE particle implant (ROI, 6 mm × 6 mm) was checked for further analysis. Bone volume to tissue volume (BV/TV) and Bone mineral density (BMD) of each sample were measured, and bone absorption pits and porosity were measured by used ImageJ software (NIH, Bethesda, Maryland, USA).

Pathological analysis of osteocytes

Recovered mice calvaria were soaked in 10% ethylenediamine tetraacetic acid (EDTA) at 4°C (pH 7.4) to achieve decalcification, then the calvaria were buried with paraffin, cut into 5 μm tissue sections, and the center of the calvaria was located at the center of the cross section. The tissue section was planned for TRAP staining, then imaged with a high-power microscope, and images were quantitatively analyzed using ImageJ software (NIH, Bethesda, Maryland, USA) to count the TRAP positive number of multinucleated osteoclasts and measure bone erosion area.

Liver and intestinal pathology analysis

As described above, the liver and small intestine of the mouse were stored in formalin, then H&E staining and TUNEL staining were used to evaluate the necrosis of liver and intestinal tissue under the microscope.

Enzyme-linked immunosorbent assay (ELISA) TEST

The blood was collected and centrifuged, then serum was collected, and the level of RANKL (receptor activator of nuclear factor κB ligand), OSCAR (osteoclast-associated receptor), CTX-1 (cross linked C-telopeptide of type I collagen) and OPG (osteoprotegerin) were determined according to the ELISA kit specification (Wuhan high technology medicine limited company in Hubei province, China).

Statistical analysis

The results were indicated by means of mean ± SD. Data were analyzed with SPSS19.0 (SPSS Inc., Chicago, IL), and the significance level was set at $P<0.05$. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test.

Results

Micro-CT

Based on the results of micro-CT and three-dimensional reconstruction, the existence of PE granules caused comprehensive osteolysis and the calvaria of the vehicle group showed more extensive bone surface erosion compared with the sham group. Interestingly, we found that the injection of tanshinone IIA significantly reduced the degree of bone damage

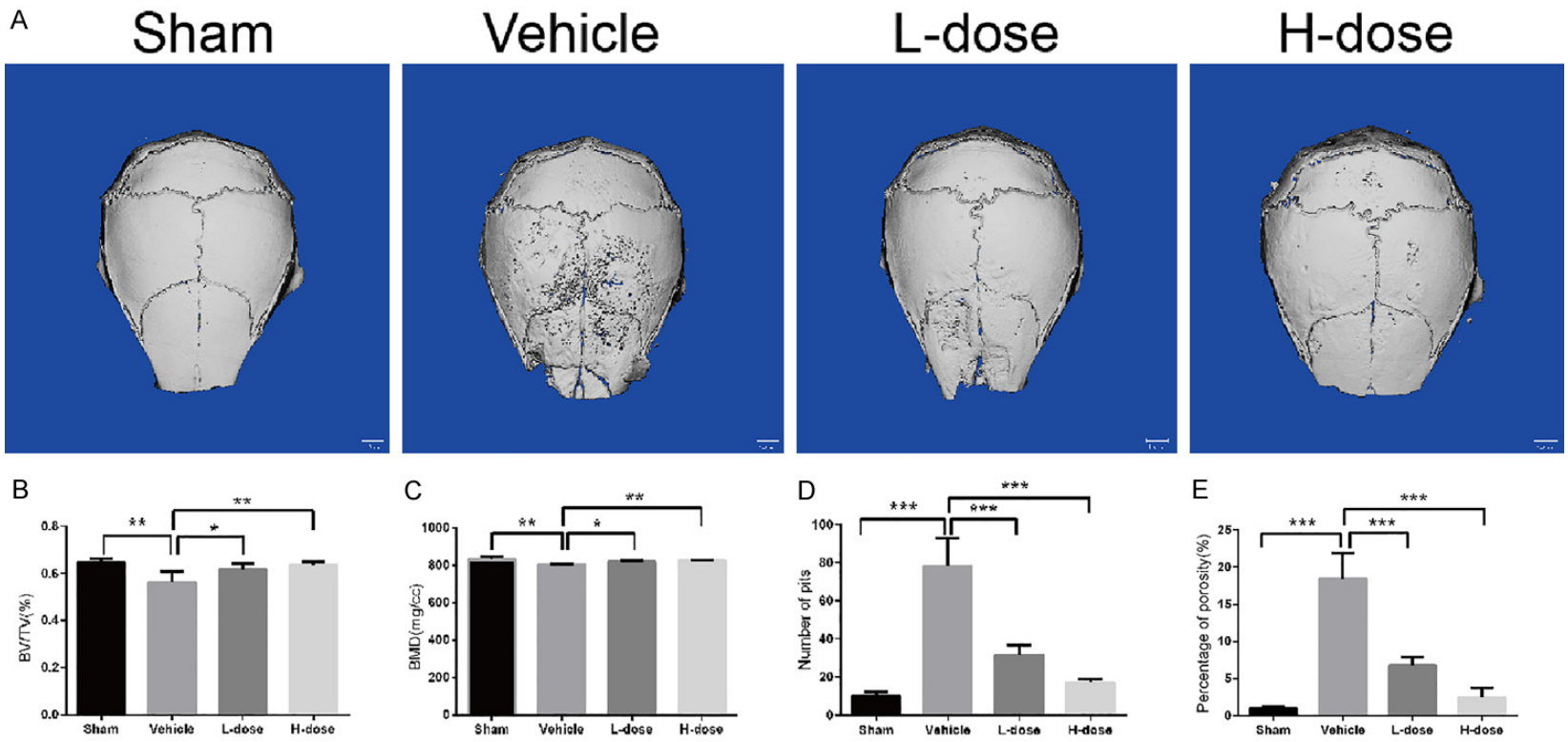

Figure 1. Tanshinone IIA prevented PE-granule-induced mice skull bone absorption. A: Typical micro-CT images of calvarial osteolysis acquired from each group. B: Bone volume to tissue volume (BV/TV). C: Bone mineral density (BMD). D: Number of pits. E: Percentage of porosity of each group was counted (n=6). *P<0.05, **P<0.01, ***P<0.001, compared with vehicle group.

induced by PE particles. Furthermore, bone loss in the higher dose group mice was markedly lower than that in lower dose group (**Figure 1A**). The quantitative bone parameters confirmed that BV/TV (**Figure 1B**), BMD (**Figure 1C**) of the calvaria induced by PE particles were significantly reduced, and the number of pits and percentage of porosity of the skull (ROI) were significantly increased (**Figure 1D** and **1E**). Thus, tanshinone IIA can effectively improve the PE particle-induced osteolysis of the calvaria.

Histomorphometric analysis of osteocytes

We next sought to explore the inhibition of tanshinone IIA on the PE particle-induced osteolysis, and to confirm that tanshinone IIA could inhibit osteoclast formation and reduce the inflammatory response. We analyzed the pathological changes of calvaria through H&E staining (**Figure 2A**) and TRAP staining (**Figure 2B**). We found that there was bone destruction in the area where PE particles were injected, and various inflammatory cells were polymerized on the bone surface of erosion, such as lymphocytes, macrophages, and most notably osteoclasts. In addition, consistent with the mCT quantification, histomorphometric results showed that the amount of TRAP (+) osteoclasts in treatment group were lower than those in the vehicle group (**Figure 2C**), which reveals the tanshinone IIA in a dose dependent manner can attenuate the formation of osteoclasts.

Tanshinone IIA inhibited the expression of RANKL and OSCAR

Our preliminary experiments have shown that tanshinone IIA can suppress the signaling pathways of RANKL-induced osteoclast formation. ELISA assay was performed to quantitatively measure the expression of RANKL and OSCAR. We found that the expression of RANKL and OSCAR in the positive group was higher than that of negative group. Furthermore, the levels of serum RANKL and OSCAR in the animals treated with tanshinone IIA were observably lower, and their concentrations were lower in higher dose group than in lower dose group (**Figure 3A**, **3B**). We suspect that the decreased expression of RANKL and OSCRA in animals treated with tanshinone IIA may explain the different amounts of differentiated osteoclasts in these two groups.

Tanshinone IIA inhibits bone absorption and reduces osteolysis

Furthermore, ELISA assay was conducted to detect CTX-1 expression in order to explore whether tanshinone IIA was effective in treating the bone damage induced by PE particles, and to evaluate whether the osteolysis was improved after the treatment of dantone IIA. These data suggested that the concentration of CTX-1 in the vehicle group was higher than that of other groups. The expression of CTX-1 can be significantly reduced after the treatment with tanshinone IIA in a dose-dependent manner (**Figure 3C**). These results indicate that tanshinone IIA could improve osteolysis induced by osteoclasts.

Tanshinone IIA can promote the expression of OPG

OPG secreted by osteoblasts is a protective factor for bony remodeling. ELISA assay was performed to measure OPG levels and quantitative analysis to determine whether the tanshinone IIA affected osteoblast expression. Interestingly, the OPG concentration of the positive group was lower than that of negative group. Compared with the vehicle group, the expression of OPG was obviously higher after tanshinone IIA treatment (**Figure 3D**). We suspect that in the process of bone destruction, tanshinone IIA regulates the balance of differentiation between osteoclasts and osteoblasts, and the expression of osteoblasts is mainly related to the inhibition of osteoclasts.

Assessing the damage to the liver and small intestine of tanshinone IIA

We next sought to confirm the metabolism of tanshinone IIA in our mouse model, and whether treatment with Tanshinone IIA caused damage to any internal organs. We extracted liver and small intestine for pathological analysis. H&E staining showed that no significant difference in liver and small intestine in each group (**Figure 4A**). TUNEL staining (**Figure 4B**) and the number of apoptotic cells (**Figure 4C**) further confirmed that there was no significant pathological difference in liver and small intestine of each group. These results showed that the tanshinone IIA did not harm the metabolism of mice.

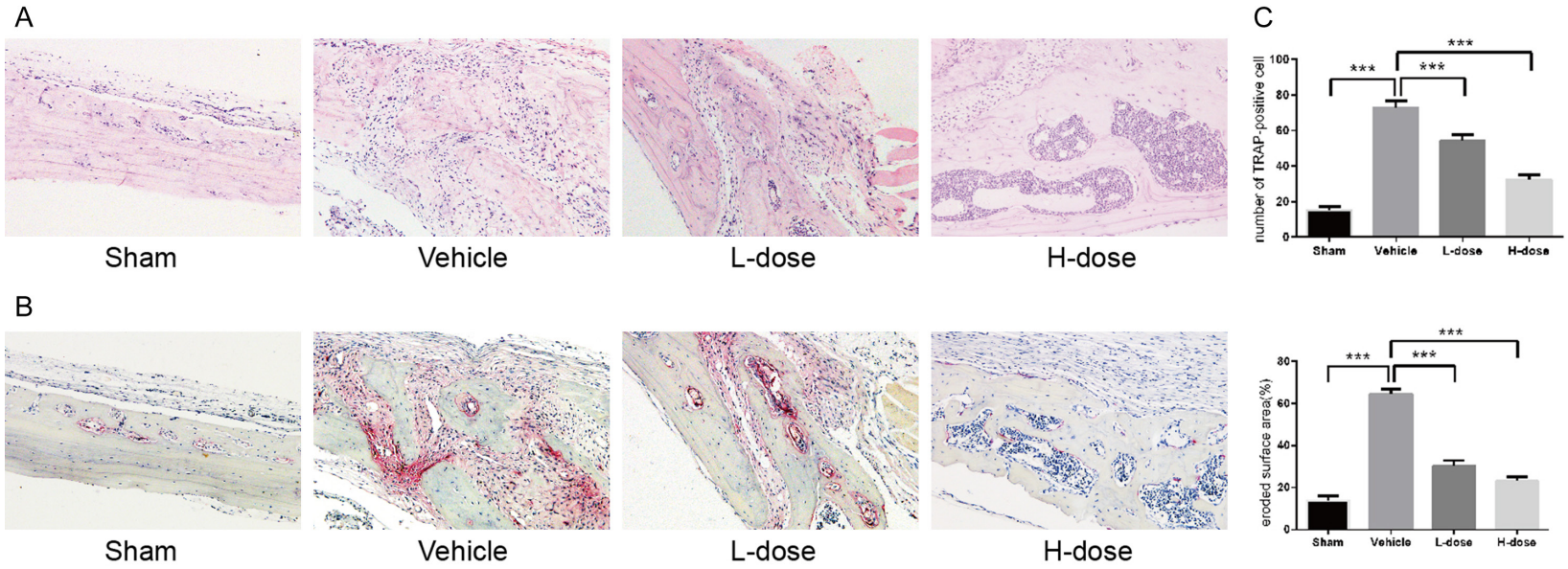

Figure 2. Histologic staining of calvarial sections. A: HE staining pictures (20 ×). B: TRAP staining pictures (20 ×). C: Quantitation of TRAP (+) osteoclasts and the erosion area of calvaria in the entire region of each group (n=6) were calculated. *P<0.05, **P<0.01, ***P<0.001, compared with vehicle group.

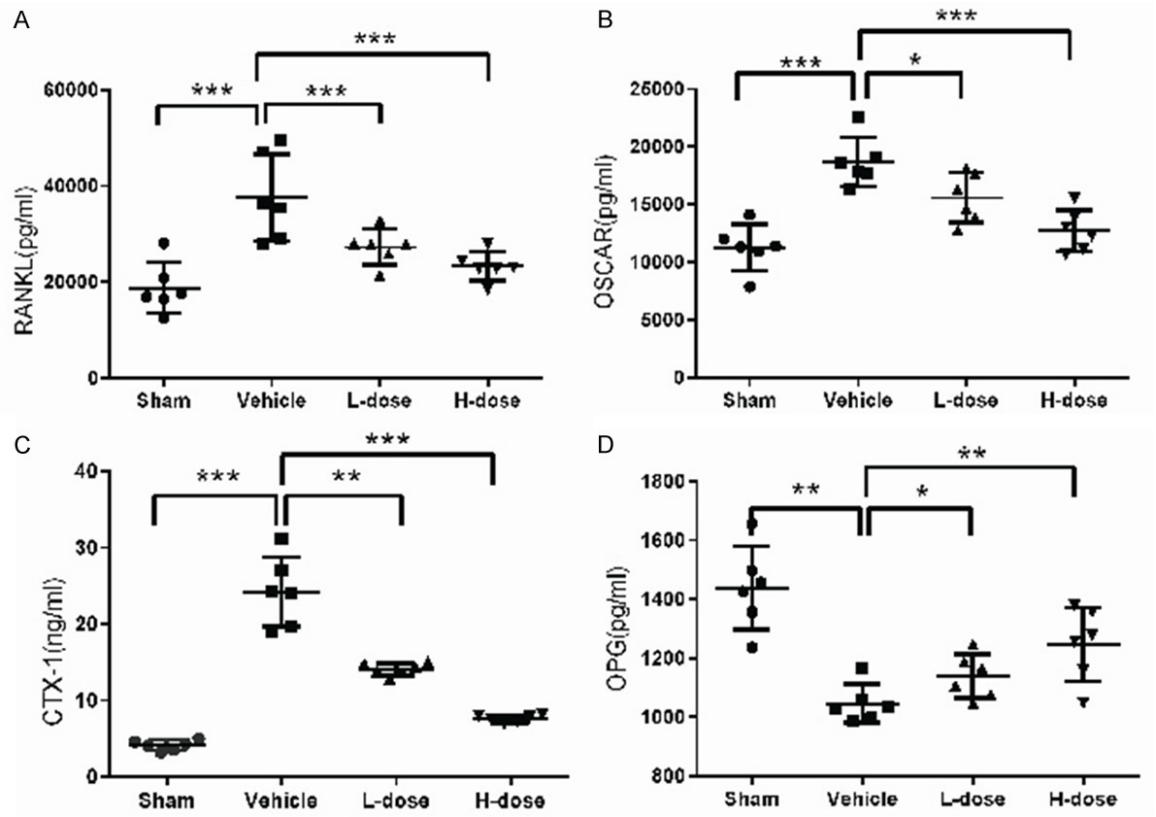

Figure 3. ELISA test results: The concentration of RANKL (A), OSCAR (B), CTX-1 (C), OPG (D) in the mouse model of PE-induced osteolysis (n=6). *P<0.05, **P<0.01, ***P<0.001, compared with vehicle group.

Discussion

Joint replacement is the most common surgical treatment of osteoarthritis. However, the mechanical wear and caused by wear particles instigates a complex biological response which limits the service life of a joint prosthesis. This is also the main reason for arthroplasty. In addition to the need for rigorous disinfection and skilled surgical techniques, we also need to work hard to improve prosthetic design and biomaterials [2-4, 31-33]. However, the production of abrasive particles is inevitable.

In addition, the development of new materials is also useless for existing osteolysis. Although the exact mechanism is unclear, but it is known that osteoclastogenesis and bone absorption of osteoclast is still an important issue in this pathologic process.

UHMWPE-induced osteolysis is the most commonly used approach for the osteolytic model. Compared with the metal particles and bone cement granules, it plays a key role in the process of bone erosion around the prosthesis [26, 29]. Similar to other studies, such as titanium particles-induced osteolysis [30, 34], we generated a mice model of cranium bone absorption by UHMWPE induced to simulate the tissue environment of prosthesis aseptic looseness. To ask whether tanshinone IIA inhibits the formation of osteoclasts by reducing the expression of RANKL, we observed and analyzed the curative effect of tanshinone IIA on bone absorption. The consequence of mCT and TRAP staining suggested that tanshinone IIA could dose-dependently inhibit the formation of osteoclasts and reduce the amounts of osteoclasts and bone resorption pits.

One explanation may be that the tanshinone IIA has significantly inhibited the formation of osteoclasts, and osteoclasts play a key role in osteolysis leaded by abrasion granules [35]. Thus, the osteoclast is considered to be a reasonable and feasible therapeutic target for the treatment of osteolysis. In recent research, it was discovered that PE granules obviously

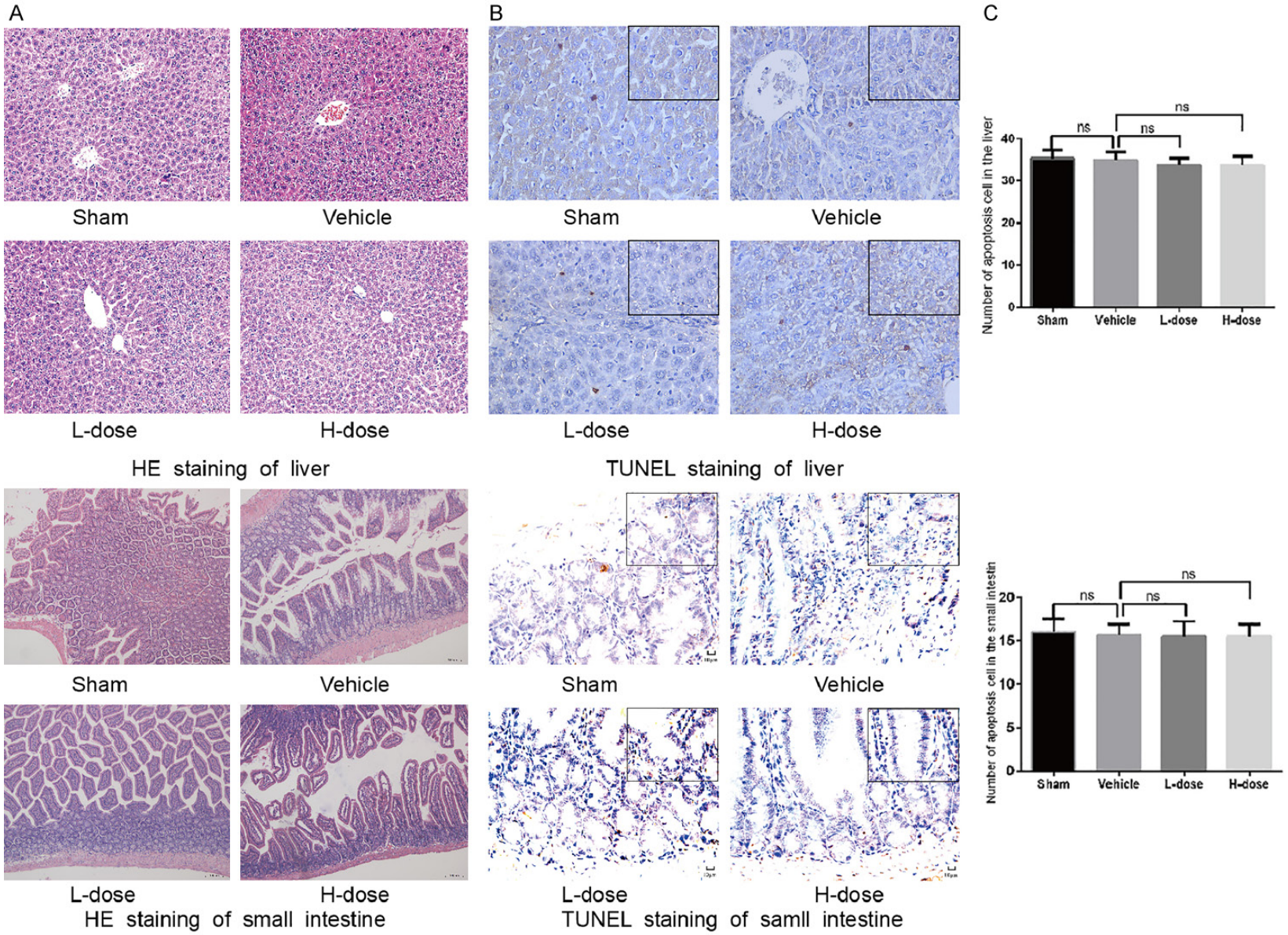

Figure 4. Groups of typical histopathologic staining images. A: H&E staining of liver (20 ×) and small intestine (10 ×). B: TUNEL staining of the liver and small intestine (40 ×). Analysis of TUNEL staining images and count the number of apoptotic cells in selected rectangular regions (image upper right). C: The number of apoptotic cells in each group (n=6). P<0.05 was considered statistically significant, ns indicates no statistical significance.

added the amount of TRAP (+) osteoclasts in the calvarial surface. Compared with the positive group, the amount of TRAP (+) osteoclasts in the mice treated with tanshinone IIA decreased significantly, which suggested that tanshinone IIA could inhibit the formation of osteoclasts. In addition, the treatment effect of tanshinone IIA on osteoporosis may be connected with the formation and differentiation of osteoclasts [22-24]. Since the formation and differentiation of osteoclasts were restrained, the area of erosion of calvarial and the amount of bone absorption pits was reduced after treatment with tanshinone IIA. These data suggest that tanshinone IIA inhibits osteoclasts formation to prevent osteolysis induced by wear particles.

Another explanation is that the guardian effect of tanshinone IIA on wear granules that lead to bone absorption may be associated with changes in the balance between OPG and RANKL. As an important regulator factor of osteoclast formation, RANKL combined with RANK can mediate the phosphorylation downstream signaling pathways such as NF-kB, NFAT and ERK [21, 36]. This signaling node promotes the formation and differentiation of osteoclasts, which play an important role in bone resorption. OPG can inhibit the combination of RANKL and RANK, thus inhibiting the bone absorption of osteoclasts. It is crucial to regulate the balance between RANKL and OPG by controlling the activation status of osteoclasts [21]. According to the results of our study, the levels of RANKL, CTX-1 and OSCAR increased, and the levels of OPG decreased in positive group compare with the sham group. Compared with the positive group, the levels of RANKL, CTX-1, OSCAR decreased and the levels of OPG increased after treatment with tanshinone IIA for 21 days. It is shown that the tanshinone IIA can prevent the osteolysis induced by wear particles by lowering RANKL and providing OPG expression.

In general, the PE particles can induce osteolysis, and tanshinone IIA has the ability to reduce RANKL-induced osteoclast formation and activate osteoclast function, so as to suppress osteolysis. The liver and small bowel pathology suggested that the administered dose of tanshinone IIA did not destroy the metabolism of the mice. Because tanshinone IIA can effectively reduce and improve the calvarial osteolysis induced by prostheses, it will provide a reliable treatment option for osteolytic disease.

There are several limitations to the current study. First, the model uses calvaria, which is formed from the internal bone of the membrane rather than the endochondral ossification. In addition, because of the lack of the implants, mechanical load and fluid pressure, there is no continuous release of PE particles, and is executed after 21 days interrupt the process of osteolysis in mice, so the clinical relevance in mouse models of calvaria should be carefully evaluated. Therefore, we need a more rigorous animal experiment to test the long-term effectiveness and safety of the tanshinone IIA. Second, bone loss in the process of osteolysis is due to the speed of bone absorption over bone formation [35]. Our results show that the treatment of tanshinone IIA can significantly improve the osteolysis induced by wear particles through inhibiting osteoclast bone absorption. It is not known whether or not tanshinone IIA can increase the differentiation and formation of osteoblasts, which may participate in the protective effect of tanshinone IIA on the induced osteolysis of wear particles. This question still needs further study.

Acknowledgements

The work was supported by the Guangxi health and family planning commission Chinese medicine science and technology project (GZLC16-32),Guangxi hygienic technology development and promotion application project (S201655) and Guangxi scientific research and technology development project (Guangxi research 1598012-41).

Disclosure of conflict of interest

None.

Address correspondence to: Jia Li, Department of Pathology, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Rd., Nanning 530021, Guangxi, P. R. China. Tel: +86-18677149533; E-mail: jialee2005@126.com; Wei Su, Department of Orthopedic, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Rd., Nanning 530021, Guangxi, P. R. China. Tel: +86-13977166802; E-mail: gxsuwei@163.com

References

[1] Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from

2005 to 2030. J Bone Joint Surg Am 2007; 89: 780-5.
[2] Johanson PE, Digas G, Herberts P, Thanner J and Kärrholm J. Highly crosslinked polyethylene does not reduce aseptic loosening in cemented THA 10-year findings of a randomized study. Clin Orthop Relat Res 2012; 470: 3083-3093.
[3] Muratoglu OK, O'Connor DO, Bragdon CR, Delaney J, Jasty M, Harris WH, Merrill E and Venugopalan P. Gradient crosslinking of UHMWPE using irradiation in molten state for total joint arthroplasty. Biomaterials 2002; 23: 717-724.
[4] Dorr LD, Wan Z, Shahrdar C, Sirianni L, Boutary M and Yun A. Clinical performance of a Durasul highly cross-linked polyethylene acetabular liner for total hip arthroplasty at five years. J Bone Joint Surg Am 2005; 87: 1816-1821.
[5] Urban RM, Hall DJ, Della VC, Wimmer MA, Jacobs JJ and Galante JO. Successful long-term fixation and progression of osteolysis associated with first-generation cementless acetabular components retrieved post mortem. J Bone Joint Surg Am 2012; 94: 1877-1885.
[6] Shin DK, Kim MH, Lee SH, Kim TH and Kim SY. Inhibitory effects of luteolin on titanium particle-induced osteolysis in a mouse model. Acta Biomater 2012; 8: 3524-3531.
[7] Jiang Y, Jia T, Wooley PH and Yang SY. Current research in the pathogenesis of aseptic implant loosening associated with particulate wear debris. Acta Orthop Belg 2013; 79: 1.
[8] Purdue PE, Koulouvaris P, Nestor BJ and Sculco TP. The central role of wear debris in periprosthetic osteolysis. HSS J 2006; 2: 102.
[9] Jiang J, Yang C, Lin Q, Yun X and Xia Y. Does arthroplasty provide better outcomes than internal fixation at mid- and long-term followup? A meta-analysis. Clin Orthop Relat Res 2015; 473: 2672-2679.
[10] Learmonth ID, Young C and Rorabeck C. The operation of the century: total hip replacement. Lancet 2007; 370: 1508-1519.
[11] Drees P, Eckardt A, Gay RE, Gay S and Huber LC. [Molecular pathways in aseptic loosening of orthopaedic endoprosthesis]. Biomed Tec 2008; 53: 93.
[12] Neuerburg C, Loer T, Mittlmeier L, Polan C, Farkas Z, Holdt LM, Utzschneider S, Schwiesau J, Grupp TM and Böcker W. Impact of vitamin E-blended UHMWPE wear particles on the osseous microenvironment in polyethylene particle-induced osteolysis. Int J Mol Med 2016; 38: 1652-1660.
[13] Noordin S and Masri B. Periprosthetic osteolysis: genetics, mechanisms and potential therapeutic interventions. Canadian J Surg 2012; 55: 408-417.
[14] Holding CA, Findlay DM, Stamenkov R, Neale SD, Lucas H, Dharmapatni AS, Callary SA, Shrestha KR, Atkins GJ and Howie DW. The correlation of RANK, RANKL and TNFalpha expression with bone loss volume and polyethylene wear debris around hip implants. Biomaterials 2006; 27: 5212-5219.
[15] Crotti TN, Smith MD, Findlay DM, Zreiqat H, Ahern MJ, Weedon H, Hatzinikolous G, Capone M, Holding C and Haynes DR. Factors regulating osteoclast formation in human tissues adjacent to peri-implant bone loss: expression of receptor activator NFkappaB, RANK ligand and osteoprotegerin. Biomaterials 2004; 25: 565-573.
[16] Kim KJ, Kotake S, Udagawa N, Ida H, Ishii M, Takei I, Kubo T and Takagi M. Osteoprotegerin inhibits in vitro mouse osteoclast formation induced by joint fluid from failed total hip arthroplasty. J Biomed Materi Res 2010; 58: 393-400.
[17] Ren W, Wu B, Peng X, Hua J, Hao HN and Wooley PH. Implant wear induces inflammation, but not osteoclastic bone resorption, in RANK-/- mice. J Orthop Res 2010; 24: 1575-1586.
[18] Granchi D, Pellacani A, Spina M, Cenni E, Savarino LM, Baldini N and Giunti A. Serum levels of osteoprotegerin and receptor activator of nuclear factor-κb ligand as markers of periprosthetic osteolysis. J Bone Joint Surg Am 2006; 88: 1501-1509.
[19] Boyce BF and Xing L. The RANKL/RANK/OPG pathway. Curr Osteop Rep 2007; 5: 98-104.
[20] Boyce BF and Xing L. Biology of RANK, RANKL, and osteoprotegerin. Arthritis Res Ther 2007; 9 Suppl 1: S1.
[21] Boyle WJ, Simonet WS and Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423: 337.
[22] Jia M, Nie Y, Cao DP, Xue YY, Wang JS, Zhao L, Rahman K, Zhang QY and Qin LP. Potential antiosteoporotic agents from plants: a comprehensive review. Evid Based Complement Alternat Med 2012; 2012: 364604.
[23] Guo Y, Li Y, Xue L, Severino RP, Gao S, Niu J, Qin LP, Zhang D and Brömme D. Salvia miltiorrhiza: an ancient Chinese herbal medicine as a source for anti-osteoporotic drugs. J Ethnopharmacol 2014; 155: 1401-1416.
[24] Kim HK, Woo ER, Lee HW, Park HR, Kim HN, Jung YK, Choi JY, Chae SW, Kim HR, Chae HJ. The correlation of Salvia miltiorrhiza extract-induced regulation of osteoclastogenesis with the amount of components tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone. Immunopharmacol Immunotoxicol 2012; 30: 347-364.
[25] Kwak HB, Yang D, Ha H, Lee JH, Kim HN, Woo ER, Lee S, Kim HH and Lee ZH. Tanshinone IIA inhibits osteoclast differentiation through down-regulation of c-Fos and NFATc1. Exp Mol Medicine 2006; 38: 256.

[26] Wedemeyer C, Xu J, Neuerburg C, Landgraeber S, Malyar NM, Von KF, Gosheger G, Von KM, Löer F and Saxler G. Particle-induced osteolysis in three-dimensional micro-computed tomography. Calcif Tissue Int 2007; 81: 394-402.

[27] Fulín P, Pokorný D, Slouf M, Vacková T, Dybal J and Sosna A. [Effect of sterilisation with formaldehyde, gamma irradiation and ethylene oxide on the properties of polyethylene joint replacement components]. Acta Chir Orthop Traumatol Cech 2014; 81: 33-39.

[28] Macdonald D, Hanzlik J, Sharkey P, Parvizi J and Kurtz SM. In vivo oxidation and surface damage in retrieved ethylene oxide-sterilized total knee arthroplasties. Clin Orthop Relat Res 2012; 470: 1826-1833.

[29] Al-Quhali AM, Sun Y, Bai X, Jin Z and Yu G. Surgical modification of the murine calvaria osteolysis model. Biomed Res Int 2015; 2015: 802697.

[30] Shao H, Shen J, Wang M, Cui J, Wang Y, Zhu S, Zhang W, Yang H, Xu Y and Geng D. Icariin protects against titanium particle-induced osteolysis and inflammatory response in a mouse calvarial model. Biomaterials 2015; 60: 92-99.

[31] Moro T, Takatori Y, Ishihara K, Konno T, Takigawa Y, Matsushita T, Chung UI, Nakamura K and Kawaguchi H. Surface grafting of artificial joints with a biocompatible polymer for preventing periprosthetic osteolysis. Nat Mater 2004; 3: 829-836.

[32] Holt G, Murnaghan C, Reilly J and Meek RM. The biology of aseptic osteolysis. Clin Orthop Relat Res 2007; 460: 240-252.

[33] Knoch FV, Heckelei A, Wedemeyer C, Saxler G, Hilken G, Henschke F, Löer F and Knoch MV. The effect of simvastatin on polyethylene particle-induced osteolysis ☆. Biomaterials 2005; 26: 3549-3555.

[34] Qiu S, Zhao F, Tang X, Pei F, Dong H, Zhu L and Guo K. Type-2 cannabinoid receptor regulates proliferation, apoptosis, differentiation, and OPG/RANKL ratio of MC3T3-E1 cells exposed to Titanium particles. Mol Cell Biochem 2015; 399: 131-141.

[35] Rao AJ, Gibon E, Ma T, Yao Z, Smith RL and Goodman SB. Revision joint replacement, wear particles, and macrophage polarization. Acta Biomater 2012; 8: 2815-2823.

[36] Asagiri M and Takayanagi H. The molecular understanding of osteoclast differentiation. Bone 2007; 40: 251-264.