Biomaterials 69 (2015) 12–21

Contents lists available at ScienceDirect

Biomaterials

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

Pharmaceutical inhibition of glycogen synthetase kinase 3 beta suppresses wear debris-induced osteolysis

Dechun Geng [a, *, 1], Jian Wu [b, 1], Hongguo Shao [a], Shijun Zhu [a], Yijun Wang [a], Wen Zhang [c], Zichuan Ping [a], Xuanyang Hu [a], Xuesong Zhu [a], Yaozeng Xu [a, **], Huilin Yang [a, ***]

[a] Department of Orthopedics, The First Affiliated Hospital of Soochow University, People's Republic of China
[b] Department of Rheumatology, The First Affiliated Hospital of Soochow University, People's Republic of China
[c] Orthopedic Institute, Soochow University, People's Republic of China

ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form
30 July 2015
Accepted 31 July 2015
Available online 3 August 2015

Keywords:
Osteolysis
Wear debris
Osteoclast
Osteoblast
Lithium chloride

ABSTRACT

Aseptic loosening is associated with the development of wear debris-induced peri-implant osteolytic bone disease caused by an increased osteoclastic bone resorption and decreased osteoblastic bone formation. However, no effective measures for the prevention and treatment of peri-implant osteolysis currently exist. The aim of this study was to determine whether lithium chloride (LiCl), a selective inhibitor of glycogen synthetase kinase 3 beta (GSK-3β), mitigates wear debris-induced osteolysis in a murine calvarial model of osteolysis. GSK-3β is activated by titanium (Ti) particles, and implantation of Ti particles on the calvarial surface in C57BL/6 mice resulted in osteolysis caused by an increase in the number of osteoclasts and a decrease in the number of osteoblasts. Mice implanted with Ti particles were gavage-fed LiCl (50 or 200 mg $kg^{-1}d^{-1}$), 6 days per week for 2 weeks. The LiCl treatment significantly inhibited GSK-3β activity and increased β-catenin and axin-2 expression in a dose-dependent manner, dramatically mitigating the Ti particle-induced suppression of osteoblast numbers and the expression of bone formation markers. Finally, we demonstrated that inhibition of GSK-3β suppresses osteoclast differentiation and reduces the severity of Ti particle-induced osteolysis. The results of this study indicate that Ti particle-induced osteolysis is partly dependent on GSK-3β and, therefore, the canonical Wnt signaling pathway. This suggests that selective inhibitors of GSK-3β such as LiCl may help prevent and treat wear debris-induced osteolysis.

1. Introduction

Total joint arthroplasty is one of the most effective treatments for severe trauma, osteoarthritis, and other arthritic diseases that affect the major joints such as the hips and knees. Aseptic loosening secondary to peri-implant osteolysis (PIO) is the leading cause of prosthesis failure and reason for revision surgery [1]. Wear debris derived from prosthetic wear accumulate at the bone-prosthesis interface and are thought to play a central role in the initiation and development of PIO [2,3]. However, the precise mechanism for this effect remains unclear. We and several other research groups have demonstrated that PIO is initiated by activation of the receptor activator of nuclear factor kappa B (RANK) and RANK ligand (RANKL) signaling pathways [4–7].

Osteoclasts play a critical role in PIO. Over the past few decades, numerous experimental and clinical evidence has demonstrated the role of osteoclasts in osteolytic disease, and numerous factors associated with the increased osteoclast activity in PIO have been identified [4,5,8,9]. To date, pharmacological interventions for wear debris-induced osteolysis have been focused on the inhibition of osteoclastogenesis [10–13]. However, even when osteoclast activity is limited, no osteoblastic repair occurs and the lytic bone fails to heal [14], suggesting that wear particles can irreversibly disrupt anabolic bone formation. Indeed, there is an increasing body of evidence demonstrating that the marked impairment in bone

* Corresponding author. Department of Orthopedics, The First Affiliated Hospital of Soochow University, 188, shi zi Road, Suzhou, 215006, People's Republic of China.
** Corresponding author. Department of Orthopedics, The First Affiliated Hospital of Soochow University, 188, shi zi Road, Suzhou, 215006, People's Republic of China.
*** Corresponding author. Department of Orthopedics, The First Affiliated Hospital of Soochow University, 188, shi zi Road, Suzhou, 215006, People's Republic of China.
E-mail addresses: szgengdc@163.com (D. Geng), xuyaozeng@163.com (Y. Xu), yhlsuzhou@126.com (H. Yang).
[1] Contribute equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2015.07.061
0142-9612/

formation caused by wear debris stimulation is a common feature in osteolytic bone disease [15–18]. This result has been confirmed in recent studies demonstrating that several markers of bone formation are decreased during wear debris-induced osteolysis [16,19]. Although the cellular and molecular mechanisms controlling this reduction in bone formation are poorly understood, the regulation of bone formation clearly plays a dominant role in the pathogenesis of PIO and is an important therapeutic target for the treatment of this destructive bone disease.

Glycogen synthase kinase 3 beta (GSK-3β), a key regulator of glycogen metabolism, is a serine/threonine kinase that plays a critical role in the regulation of bone mass [20–22]. In fact, the inhibition of GSK-3β activity by pharmacological inhibitors, such as lithium, increases bone formation [20,22]. Furthermore, the inhibition of GSK-3β is necessary for effective canonical Wnt pathway signaling, and such signals are potential candidates for osteoinductive therapies to treat osteolytic disease [23]. However, a number of studies have suggested that GSK-3β indirectly promotes osteoclasts by enhancing RANKL expression while decreasing osteoprotegerin (OPG) secretion by osteoblasts [24,25]. The balance between RANKL and OPG is an important determinant for controlling bone mass in both normal and diseased states. Together, these data indicate that GSK-3β is essential for the regulation of bone remodeling. However, little information on the role of GSK-3β in wear debris-induced osteolysis is available. The purpose of this study was to determine the role that GSK-3β plays in the process of PIO. We hypothesized that pharmaceutical inhibition of GSK-3β prevents wear debris-induced osteolysis by accelerating bone formation and reducing bone resorption.

2. Materials and methods

2.1. Preparation of titanium particles

Commercially available pure titanium (Ti) particles were obtained from Johnson Matthey (#00681; Ward Hill, MA, USA). According to the manufacturer, 90% of the Ti particles were < 3.6 μm in diameter, which is clinically relevant size range. The Ti particles were prepared as previously described [4,6,12], and a commercial limulus amebocyte lysate assay kit (QCL-1000; Biowhittaker, Walkersville, MD, USA) was used to confirm that the Ti particles were free of endotoxin. The Ti particles were sonicated and vortexed before use.

2.2. Experimental animals

All animals were treated according to the principles and procedures approved by the First Affiliated Hospital of Soochow University. Eighty-four female 6- to 7-week-old C57BL/6 mice were obtained from the Laboratory Animal Research Center of Soochow University. Each of the mice weighed 20–25 g at the beginning of the study.

2.3. Calvarial osteolysis model and drug treatment

The murine calvarial osteolysis model was created as previously described [11–13,26]. Briefly, the mice were randomly divided among four experimental groups: a control group receiving no Ti particles (control), a Ti particle group (Ti), and two groups administered Ti particles together with LiCl at either a low (L-LiCl) or high (H–LiCl) level. The mice were anesthetized with an intraperitoneal injection containing 50 mg kg^{-1} of pentobarbital. Either no Ti particles (control group) or 20 mg of Ti particles (Ti, L-LiCl, and H–LiCl groups) were placed directly on the surface of the calvarial bone. Before surgery, all mice received a subcutaneous injection of Carprofen (4 mg kg^{-1}; KDN PHARM, Qingdao, China), and the oral antibiotic Enrofloxacin (100 mg mL^{-1}; GuideChem, Nanjing, China) was administered in the drinking water for 3 days after the operation. The mice in the L-LiCl and H–LiCl groups were gavage fed the GSK-3β selective inhibitor lithium chloride (50 or 200 mg $kg^{-1}d^{-1}$; Sigma, St. Louis, MO, USA) or 300 μL of distilled water (control and Ti groups) 6 days per week for 2 weeks. The oral dosage used here is effective for the treatment of human bipolar illness, and has been shown to increase the bone mass in mice [20,27]. Blood samples were collected from the mice just prior to sacrifice at 2 weeks after the operation, and the calvariae were dissected for molecular, micro-computed tomography (μCT), and histological analyses.

2.4. Micro-computed tomography analysis

The dissected calvariae were scanned with a high-resolution μCT scanner (SkyScan 1176; SkyScan, Kontich, Belgium) as previously described [28]. The skulls were scanned at a resolution of 18 μm operating at a source voltage of 80 kV and 100 μA with an exposure time of 100 ms. After scanning, three-dimensional (3D) images were reconstructed using the manufacturer's software. For quantitative analysis of the particle-induced osteolysis, CT Analyzer software (CT An, SkyScan) was used after selecting a cylindrical region of interest (ROI; 3 × 3 × 1 mm) centered around the intersection of the sagittal and coronal sutures [28]. Based on the morphology of the cortical bone, bone volume to tissue volume ratio (BV/TV), bone mineral density (BMD, mg mm^{-2}), and number of pores within the ROI were obtained as previously described [11,12,28].

2.5. Bone histomorphometry

After μCT scanning, the calvaria were decalcified in 10% EDTA for 3 weeks and then embedded in paraffin using standard procedures. The samples were sectioned to 4–5 μm, mounted on protein-coated glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E). Using a magnification of 20 × , digital photographs centered on the midline suture of the sections were taken. The region of interest (ROI) was defined as previously recommended [11,28]. Histomorphometric analysis was performed using Image Pro Plus software 6.0 (Media Cybernetics, Silver Spring, MD, USA). The eroded bone surface (EBS, mm^2) in the slices, as determined by H&E staining, was evaluated as described previously [9]. To determine bone thickness (BT, mm), the sections were divided into four 0.5-mm sub-regions to the left and four to the right side of the midline suture [29]. The BT was measured in these regions and at the midline suture.

To detect osteoclasts, the sections were stained for tartrate-resistant acid phosphatase (TRAP) using a commercial kit. Dark purple-stained multinucleated cells located on the bone perimeter within a resorption lacuna were considered as TRAP-positive cells. Positive TRAP localization was quantified by counting the pixel area in the region of interest in five consecutive calvaria sections. In addition, the percentage of osteoclast surface per bone surface (OcS/BS, %) was calculated as previously described [11,12].

For immunohistological staining, the deparaffinized sections were incubated with a primary antibody overnight at 4 °C. The primary antibodies used were the GSK-3β antibody (1:200, ab32391, Abcam, Shanghai, China), the phosphor-Ser9-GSK-3β antibody (1:250, ab75814, Abcam), the β-catenin antibody (1:500, ab32572, Abcam), alkaline phosphatase (ALP) antibody (1:500, ab108337, Abcam), the osterix antibody (1:100, ab22552, Abcam), the OPG antibody (1:200, ab9986, Abcam), and the RANKL antibody (1:500, ab9957, Abcam). After washing, the sections were incubated with a biotin-conjugated secondary antibody for 30 min,