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The effect of enoxacin on osteoclastogenesis and reduction of titanium particle-induced osteolysis via suppression of JNK signaling pathway

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ABSTRACT

The aim of this study was to assess the effect of enoxacin on osteoclastogenesis and titanium particle-induced osteolysis. Wear particles liberated from the surface of prostheses are associated with aseptic prosthetic loosening. It is well established that wear particles induce inflammation, and that extensive osteoclastogenesis plays a critical role in peri-implant osteolysis and subsequent prosthetic loosening. Therefore, inhibiting extensive osteoclast formation and bone resorption could be a potential therapeutic target to prevent prosthetic loosening. In this study, we demonstrated that enoxacin, a fluoroquinolone antibiotic, exerts potent inhibitory effects on titanium particle-induced osteolysis in a mouse calvarial model. Interestingly, the number of mature osteoclasts decreased after treatment with enoxacin *in vivo*, suggesting that osteoclast formation might be inhibited by enoxacin. We then performed *in vitro* studies to confirm our hypothesis and revealed the mechanism of action of enoxacin. Enoxacin inhibited osteoclast formation by specifically abrogating RANKL-induced JNK signaling. Collectively, these results suggest that enoxacin, an antibiotic with few side effects that is widely used in clinics, had significant potential for the treatment of particle-induced peri-implant osteolysis and other diseases caused by excessive osteoclast formation and function.

1. Introduction

Total joint arthroplasty (TJA) is thought to be the most effective treatment for severe trauma, osteoporotic fracture, and arthritic joint diseases. Approximately 1.5 million cases of TJA are performed annually worldwide [1]. Although much effort has been made to improve the efficacy of TJA, particle-induced periprosthetic osteolysis and subsequent aseptic loosening continue to be major causes of arthroplasty failure [2,3]. Wear particles liberated from the surface of prostheses play a critical role in osteolysis [4]. Although the underlying mechanisms of particle-induced osteolysis remain unclear, studies suggest that osteolysis at the periprosthetic site results from excessive osteoclast activation, which is initiated by activation of the receptor activator of the nuclear factor-κB (RANK) and RANK ligand (RANKL) signaling pathways [5,6]. Generally, the wear particles stimulate macrophages, phagocytes, fibroblasts, and T lymphocytes to secrete high concentrations of cytokines and chemokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-11, and IL-17, prostaglandin E_2 (PGE_2), and macrophage-colony stimulating factor (M-CSF). This can result in the overexpression of RANKL in osteoblasts, which can subsequently stimulate excessive osteoclast formation [7–9]. Due to the importance of osteoclasts in this osteolytic disease, inhibitors that can suppress osteoclast formation and/or function have great potential to prevent wear particle-induced osteolysis and pathological bone loss [10].

Enoxacin, a fluoroquinolone antibiotic, is widely used to treat patients with urinary tract infections and gonorrhea. Recently, unexpected properties of enoxacin were reported. Enoxacin was

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found to inhibit osteoclast formation and function by interfering the interaction between the V-ATPase B2-subunit and microfilaments or by blocking the interaction between V-ATPase a3-subunit and microfilaments [11,12]. However, it remains unclear whether enoxacin can prevent osteolytic diseases *in vivo*. To investigate this, we established a mouse model of titanium particle-induced osteolysis and showed that enoxacin could protect against particle-induced osteolysis. Surprisingly, osteoclast formation was inhibited in enoxacin-treated mice *in vivo*. Therefore, we hypothesized that enoxacin inhibited both osteoclast function and formation. To test this hypothesis, we performed a series of biochemical and morphological assays *in vitro*. Enoxacin specifically suppressed JNK phosphorylation and inhibited RANKL-induced JNK signaling, which plays a critical role in osteoclast formation [13–15]. This suggests that enoxacin, a widely used potent antibiotic with few side effects, has the potential to be translated into an agent for the treatment of diseases caused by excessive osteoclast formation and function, including particle-induced peri-implant osteolysis.

2. Results

2.1. Effect of enoxacin on titanium particle-induced osteolysis

Previous studies had demonstrated that enoxacin could inhibit the interaction between V-ATPase subunit B2/V-ATPase subunit a3

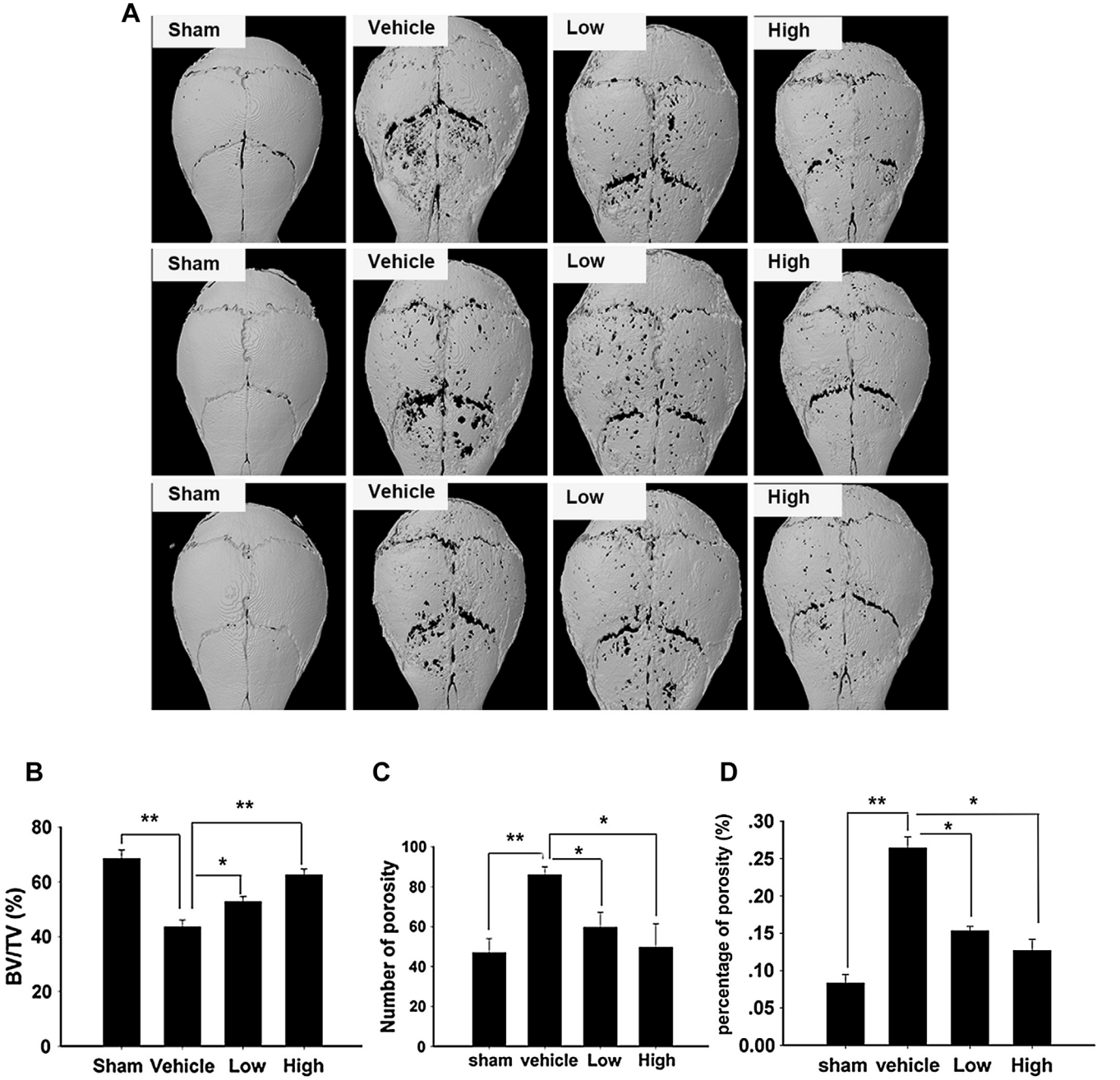

Fig. 1. Enoxacin (Eno) prevented titanium particle-induced mouse calvarial osteolysis. (A) Representative micro-computed tomography (CT) three-dimensional reconstructed images from each group. (B) Bone volume against tissue volume (BV/TV), number of porosity, and the percentage of total porosity of each sample was measured (*$P < 0.05$; **$P < 0.01$).