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The JNK signaling pathway against titanium-particle-induced osteoclastogenesis and bone resorption *in vivo*

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Abstract. – OBJECTIVE: The c-Jun N-terminal kinases (JNK) signaling pathway may be involved in the regulation of osteoclast development. The purpose of this investigation was to investigate whether SB600125, a JNK inhibitor, could attenuate titanium-particle-induced inflammatory osteolysis in vivo.

MATERIALS AND METHODS: A total of 45 mice were randomly divided into a Sham group, a Titanium group, and a Titanium + JNK inhibitor group, 15 mice per group. After establishing an air pouch bone graft model, we injected phosphate-buffered saline (PBS), titanium particles, or titanium particles + JNK inhibitor into the air pouch of the three groups. The pouch membranes containing bone implants were taken for morphological and molecular analysis 14 days after the mice were sacrificed.

RESULTS: General morphological structure observation results, Hematoxylin and Eosin (H&E)-Stained Sections, anti-tartaric acid phosphatase (TRAP) staining, and the transmission electron microscope showed that SB600125, by inhibiting the expression of JNK, attenuated titanium particle-induced inflammatory osteolysis ($p<0.05$). Immunohistochemical appearance results and reverse transcription-polymerase chain reaction (RT-PCR) results showed SB600125 reduced expression of IL-6, and TNF-α in osteolytic sites stimulated with wear debris ($p<0.05$). The Western blot results showed the expression of the p-JNK protein in the titanium particle + SB600125 group was significantly reduced compared to the titanium particle stimulation group ($p<0.05$).

CONCLUSIONS: Interfering with the JNK signaling pathway may be beneficial in reducing osteolysis, providing a therapeutic target for preventing and treating aseptic loosening caused by debris-induced inflammatory osteolysis.

Key Words:
Wear particles, Inflammatory, Osteolysis, c-Jun N-terminal kinases, Aseptic loosening.

Introduction

Preventing prosthesis loosening, an important complication that affects the quality of life of patients after a joint replacement has become essential in prolonging the lifespan of a prosthesis[1]. Thus, new therapeutic drugs and methods need to be identified. The titanium debris generated at the bone-implant interface is a significant contributor to the localization of osteoclastic and inflammatory peri-prosthetic tissue damage as well as bone resorption[2-4]. Additionally, the titanium debris induces fibroblasts and macrophages to congregate in the interfacial membranes, which activates the cells and causes them to release a lot of inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), metalloproteinase (MMP)-9, and others. The process of titanium debris-induced inflammatory osteolysis is greatly influenced by these variables[5,6].

The JNK family is a member of the mitogen-activated protein kinases (MAPKs) superfamily, which is an important branch of the

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MAPK signaling pathway. Currently, JNK protein kinases are encoded by three genes in mammalian cells: JNK1, JNK2, and JNK3[7]. The JNK signaling pathway can be activated by a variety of factors, including cytokines, growth factors, stress, etc. The JNK signaling pathway regulates the cell cycle in physiological and pathological states as well as cellular stress responses in specific situations, and is involved in the pathological response to many diseases. The JNK signaling pathway modulates the expression of pro-inflammatory cytokines like IL-1, IL-6, and cyclo-oxygenase-2 (COX-2), which are significant mediators of acute and chronic inflammation, the foreign body response, peri-prosthetic osteolysis, and apoptosis. However, studies[8] on the JNK signaling pathway are lacking and limited to the cellular level, and the results of animal experiments are lacking. JNK signaling pathway is widely thought to be inhibited by the pyridinyl imidazole SB600125[9]. Therefore, in this study, we established a wear particle-induced osteolysis model in mice (mouse air pouch osteolysis model) and investigated the role of the JNK signaling pathway in osteoclasts using the JNK signaling pathway inhibitor SP600125, which can help to understand better the pathogenesis of periprosthetic osteolysis after artificial joint replacement. To expand new ideas for the prevention and treatment of aseptic loosening due to periprosthetic osteolysis after prosthetic arthroplasty, and to address the pain caused by revision to patients.

Materials and Methods

Titanium Processing

The Alfa Aesar company manufactured titanium (Alfa Aesar, Ward Hill, MA, USA). Titanium was measured using a scanning electron microscope (Hitachi FESEM S-4800, Hitachi, Kyoto, Japan), and the majority of the particles were less than 10 μm, which is the most typical clinical size range (Figure 1). The particles were washed in 70% ethanol solution for 48 hours to eliminate any bound endotoxins after being sterilized at 180°C for 6 hours. The Limulus Amebocyte Lysate test was used to detect whether endotoxins were present. The particles were suspended in sterile phosphate-buffered saline (PBS) at a concentration of 5% (weight/volume) and kept at 4°C after being rinsed three times with sterile PBS.

Air Pouch Osteolysis Model Construction

All research involving animals was performed in accordance with the standards for the Care and Use of Laboratory Animals, and it was all approved by the Ningxia Medical University Animal Care Committee (No: 2015-019). Each experiment complied with the Helsinki Declaration and was carried out in accordance with accepted ethical standards for using animals in research. Weight: 20-24 g, female BALB/c mice, 8-10 weeks old. The air pouch osteolysis model construction was made using Chen's method[10]. Sham group, titanium group, and titanium + JNK inhibitor group were the three experimental groups to which a total of 45 mice were randomly assigned. Each group contained fifteen mice. The pouches of the last two groups received 0.3 ml of a suspension containing 5% (weight/volume) titanium the day after the surgical procedure, while the pouches of the Sham group received 0.3 ml of PBS. The titanium + JNK inhibitor group was subsequently given 0.1 mg/kg/day of SB600125 intraperitoneally, and the Sham and titanium group received 0.1 ml of sterile PBS intraperitoneally. 14 days after the bone implantation, mice were sacrificed. The bone implants and pouch membranes were collected for morphological and molecular analyses.

Hematoxylin and Eosin (H&E) Staining

4% polyoxymethylene with a pH of 7.4 was used to fix tissue specimens for 24 hours. The specimens were processed for dehydrating in graded alcohols, clearing in dimethyl benzene, and embedding in paraffin after being decalcified in 10% ethylenediaminetetraacetic acid for 4

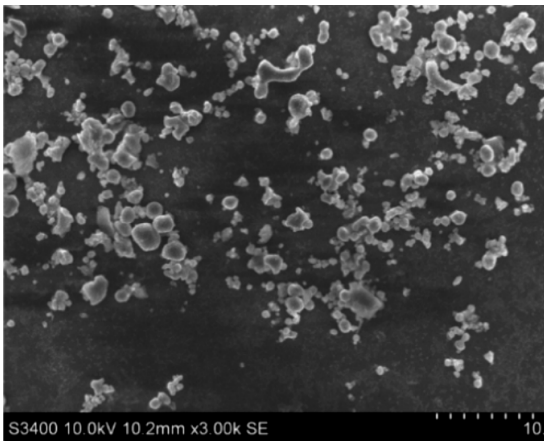

Figure 1. Scanning electron microscopy appearance of the titanium particles (magnification, 3000×).

weeks. H&E staining of tissue slices (6 μm) was used to evaluate pouch membrane inflammation and implant-bone erosion. A light microscope was used to view the stained slices in order to see how the surface of the air pouch and the area around the bone graft had changed.

Tartrate-Resistant Acid Phosphatase (TRAP) Staining

After the skull was sliced, it was dewaxed and dehydrated and then naturally dried. This experiment used the American GenMed paraffin section TRAP immunohistochemical staining kit, strictly following the manufacturer's step-by-step instructions, and used Image J software to evaluate the number of positive cells.

Transmission Electron Microscope

The mouse skull and its pouch membrane tissue were trimmed for fixation (0.5 cm × 0.3 cm × 0.3 cm). The specimens were then fixed in 1% osmic acid fixation solution for 2 hours after being rinsed three times in sodium arsenic buffer. After that, they were rinsed twice with various doses of dimethyl sodium arsenic buffer at 20-minutes intervals. The resin was immersed in an oven at 42°C for the night after being treated with propylene oxide penetration and alcohol dehydration. Under an optical microscope, continuous ultra-thin sectioning was performed, and the slices were laid out on a copper mesh covered in formvar film. The sample rod was inserted, and we examined the slices.

Immunohistochemistry Staining for IL-6 and TNF-α

Paraffin-embedded sections were treated with 3% hydrogen peroxide applied dropwise to inactivate endogenous peroxides for the immunohistochemical staining of IL-6 and TNF-α and then heated in a microwave to conduct antigen retrieval. The sections were then stained with rabbit anti-mouse primary antibodies (1: 500; all acquired from Abcam, Cambridge, MA, USA) at 4°C for 8 hours after the antigen had been blocked for 10 min with 5% goat serum. After a PBS wash, the sections were incubated for 30 minutes with goat anti-rabbit IgG (Proteintech Group, Chicago, IL, USA) before being colored with 3,3′-diaminobenzidine (DAB) dye solution, which was applied dropwise. The slides were mounted, dehydrated, clear, and counterstained with hematoxylin, photographed and analyzed using Image J analysis software.

Gene Expression of IL-6 and TNF-α

We randomly selected 3 mice from each group, took out the implanted skull and pouch membrane tissues, shredded and ground them thoroughly under the action of liquid nitrogen. Then, we added 1 ml of TRIzol (Invitrogen, Chicago, IL, USA) and extracted the RNA according to the manufactory's instructions, followed by RNA reverse transcription to synthesize cDNA at 37°C 60 minutes and 95°C 5 minutes and stored the cDNA at −80°C. We performed PCR amplification according to the instructions of the fluorescence quantitative PCR kit, and the amplification conditions were 95°C preheated for 2 minutes, denatured at 94°C for 15 seconds and annealed at 65°C for 1 minute for a total of 40 cycles. Each specimen was analysed in triplicate. The primer sequences used were as follows: *IL-6*, forward 5′- TACTGGAAGATGTCGTGTGAG-3′ and reverse 5′-GGCGTGTCTGGAGATTCG-3′; *TNF-α*, forward 5′-CAGGTCACTGTGTCCCAGCATCT-3′and reverse 5′-GAGTCCGGGCAGGTCTACTTT-3′; *Gapdh*, forward 5′-TGCTGTTGAAGTCGCAGGAC-3′ and reverse 5′-CCAATGTGTCCGTCGTGGAT-3′. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The relative expression of mRNA was calculated using the $2^{-\Delta\Delta CT}$ method.

Western Blot Analysis of JNK and p-JNK

Specimens were manually homogenized in protein extraction buffer after being chopped into small pieces. Protein concentration was determined using the DC Protein Assay Kit™ (BioRad, Hercules, CA, USA) and 60 μg protein was separated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto nitrocellulose membranes. Following a 1-hour block of the membranes. Primary antibodies (JNK, 1:1000; p-JNK, 1: 800; β-actin, 1: 2 000; Cell Signaling Technology, Chicago, IL, USA) were incubated with the membrane for an overnight at 4°C. The membrane was incubated with Horseradish Peroxidase (HRP)-antirabbit secondary antibody (1: 5000; Proteintech) for 1-hour the next day after being rinsed three times with Phosphate Buffered Saline with Tween20, (PBST). With the use of BIO-RAD scanning equipment, the bands could be seen. The internal reference was β-actin.

Statistical Analysis

Data processing and statistical analysis used the SPSS statistical package, version 11.0 (SPSS

Inc., Chicago, IL, USA). The data are expressed as the mean±standard error of the mean (SE). Measurement data used analysis of Student's *t*-test and one-way ANOVA tests. Differences were considered at the significance level of $p<0.05$. The study was carried out in compliance with the ARRIVE guidelines.

Results

Morphological Structure Observation

The air pouch membrane tissue is harvested and observed. In the Sham group, the membrane enclosed the implanted skull tissue, and there was no obvious capillary proliferation around it and the inflammatory response was minimal (Figure 2A). In the Titanium group, the membrane was tightly wrapped in titanium particles, and in the implanted skull, the membrane was thickened, there was clear capillary proliferation around the wall, and inflammation was manifested (Figure 2B). In the Titanium + JNK inhibitor group, the membrane was wrapped in titanium particles and the implanted skull tissue, but compared with the Titanium group, the surrounding capillary proliferation was relatively reduced, and the inflammation was reduced (Figure 2C). Cellular infiltration and air pouch thickness were evaluated by histological analysis. Bone erosion was significantly higher in the titanium particle-induced bone-implanted pouches compared to the Sham group, particularly at the bone-membrane interface area where erosion pits were seen. The inflammatory osteolytic reaction in the pouches that were implanted in the skull decreased after treatment with SB600125, and the surface of the skull was comparatively smooth (Figure 3).

Effect of SB600125 on Ti Particle-Induced Osteoclastogenesis

An increase of TRAP-positive cells can be seen at the interface of the implanted skull and the pouch membrane. Few TRAP-positive cells were seen in the Sham group skull-implanted pouches without titanium particle activation. In contrast, skull-implanted pouches activated by titanium particles had a substantial rise in TRAP-positive staining. The Titanium + JNK inhibitor group displayed a reduction in the number of TRAP-positive osteoclast-like cells (Figure 4). The statistical results are as follows. The numberof TRAP staining positive cells (14.530±2.615) in the Titanium group was significantly higher than that in the Sham group (4.867 ± 1.642) ($p<0.05$), and the number of TRAP staining positive cells in the Titanium + JNK inhibitor group (9.467 ± 2.386) was greatly reduced compared with that in the Titanium group (14.530±2.615) ($p<0.05$).

Analysis of the Transmission Electron Microscope

In this experiment, it was shown that the Sham group had fewer osteoclasts and smaller nuclei. The cytoplasmic organelles showed no outward signs of abnormality, and there were not many lysosomes. The number of osteolytic cells was much higher in the Titanium group, the nuclei were larger, the cytoplasm was plentiful, and there were considerable numbers of rough

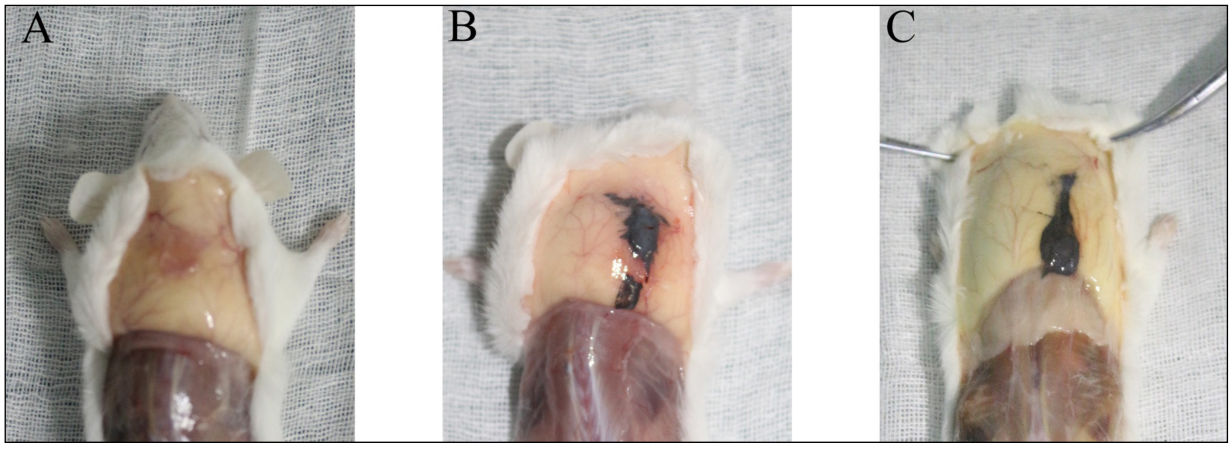

Figure 2. Morphological structure observation. Air pouches in the Sham (**A**), Titanium (**B**), and Titanium + JNK inhibitor groups (**C**). The position indicated by the blue arrow is the implanted skull or (and) Ti particles.

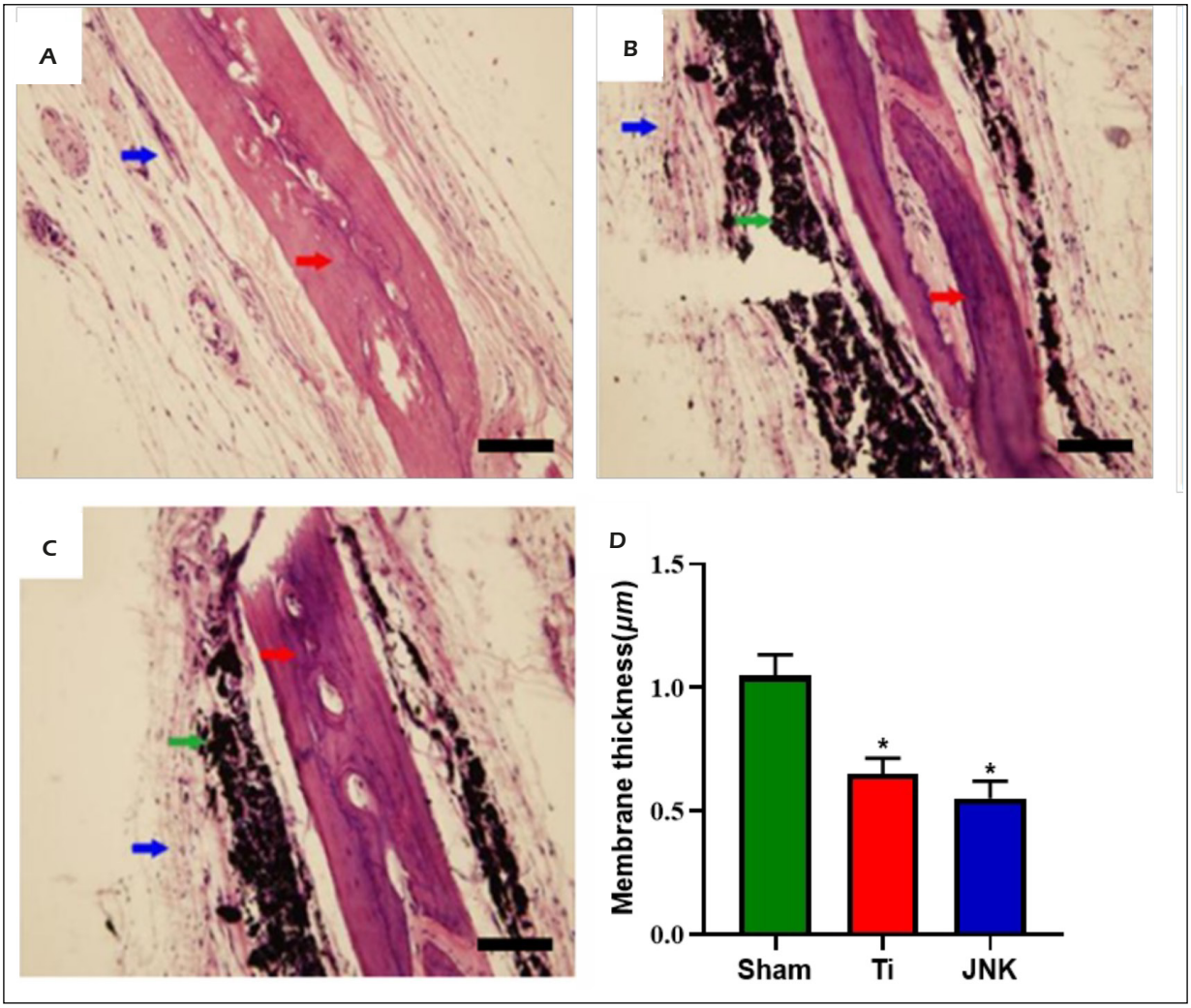

Figure 3. Histological of air pouches after H&E staining (×40). **A**, is the Sham group. **B**, Is the Titanium group, and (**C**) is the titanium + JNK inhibitor group. The blue arrows refer to the pouch membrane around the implanted skull, the green arrows refer to the implanted titanium particles, and the red arrows refer to the implanted skull. **D**, *p<0.05 compared with the Sham group; n=5 per group.

endoplasmic reticulum and mitochondria in the cytoplasm. On the outside of the bone tissue, osteolytic alterations can be noticed. The rough endoplasmic reticulum, lysosomes, and mitochondria were all considerably diminished, and osteoclast activity was impaired in the Titanium + JNK inhibitor group (Figure 5).

Immunohistochemical Analysis of IL-6 and TNF-α

Proinflammatory cytokines are believed to contribute to bone resorption through the promotion of osteoclast differentiation. Thus, we evaluated the expression of the following cytokines associated with osteoclastogenesis: IL-6 and TNF-α. Immunohistochemical analysis revealed that Titanium particles drastically increased the expression of IL-6 and TNF-α in the bone-graft samples. However, The Titanium + JNK inhibitor group significantly decreased the expression of IL-6 and TNF-α (Figures 6 and 7).

Gene Expression of IL-6 and TNF-α

With this analysis of the RT-PCR results, significantly increased gene expression of *IL-6* and *TNF-α* was detected in the Titanium group compared with the Sham controls (p<0.05). SB600125 treatment resulted in a remarkable reduction in the expression of these genes compared to the titanium-stimulated bone-implanted pouches (p<0.05) (Figure 8).

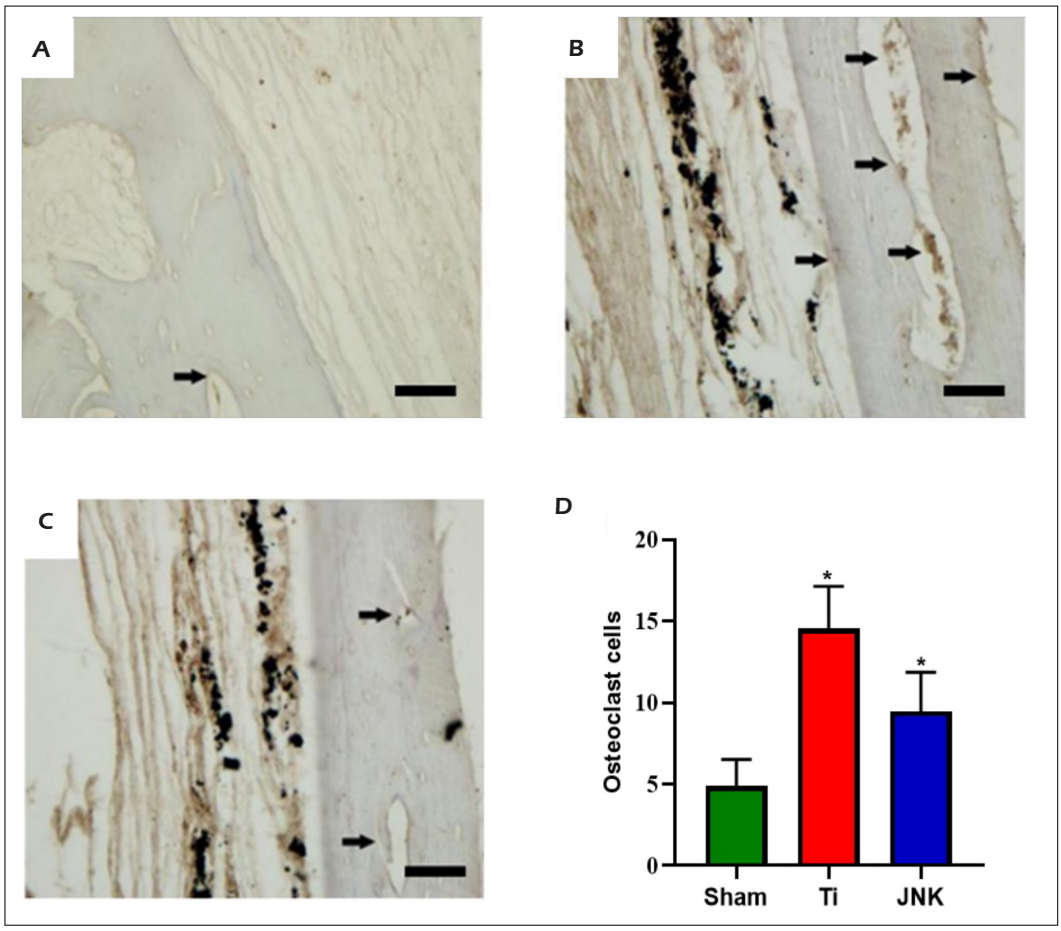

Figure 4. Representative TRAP staining images (×40). **A**, Skull-containing pouch with PBS injection (Sham). **B**, Demonstrates a skull-containing pouch with Ti particle stimulation (titanium group). An extensive dark purple stain along the pouch membranes shows the deposits of osteoclast-like cells. **C**, Skull-containing pouch with titanium particles+SB600125 treatment (titanium + JNK inhibitor group). **D**, **p*<0.05 compared with the Sham group; n=5 per group.

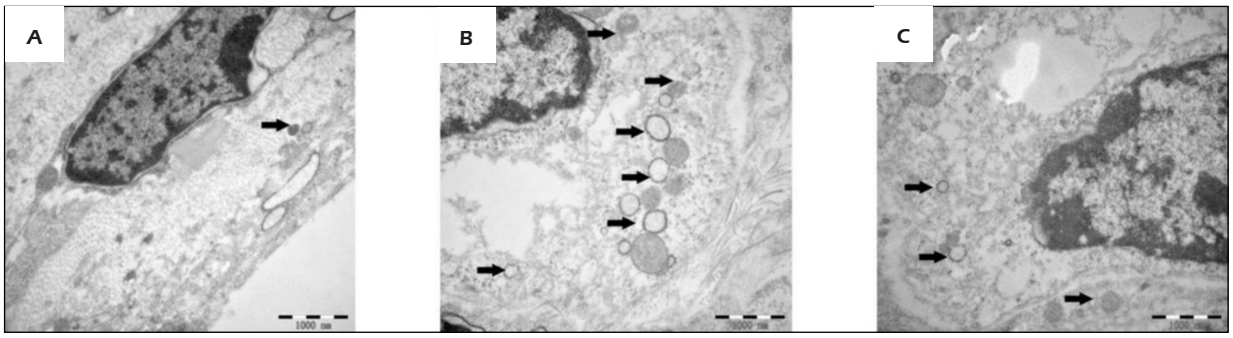

Figure 5. Three groups of transmission electron microscope results (×300). **A**, Is the Sham group. **B**, Is the Titanium group, and (**C**) is the Titanium + JNK inhibitor group. Different numbers of osteoclasts can be seen in the three groups of skull tissue, as shown by the black arrows (n=5 per group).

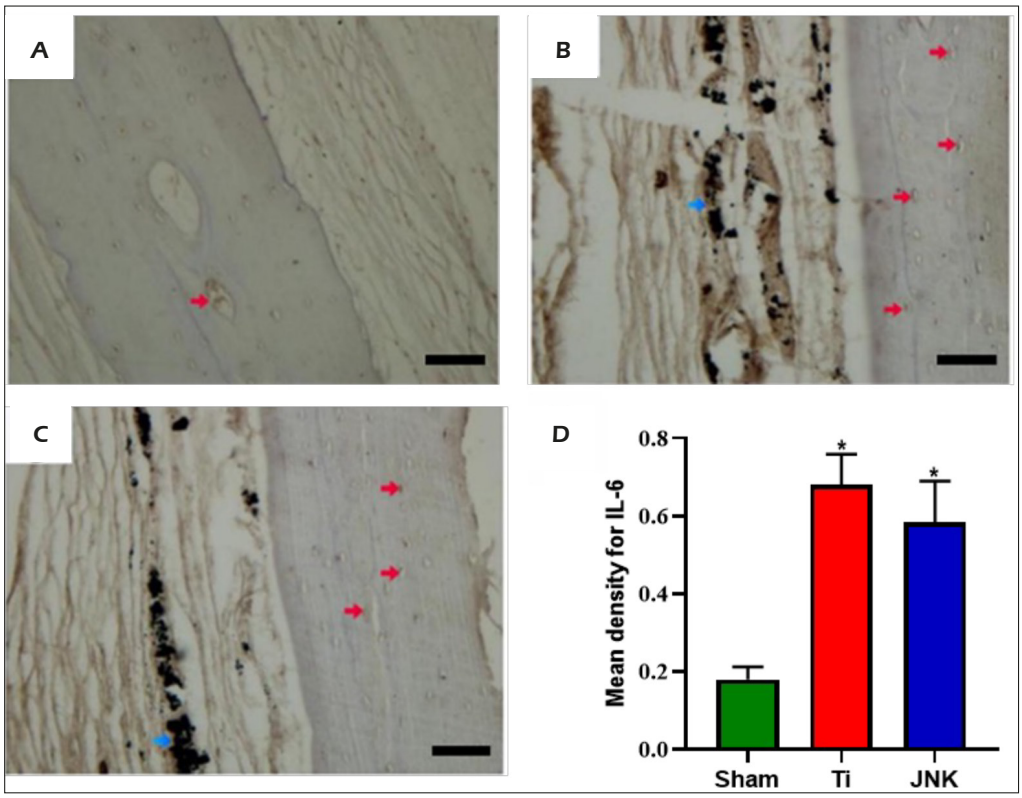

Figure 6. Immunohistochemical detection of IL-6 (×200). Air pouches in the Sham (**A**), Titanium (**B**) and Titanium + JNK inhibitor group (**C**). Titanium particles stimulated increased immunohistochemical positive cells, while SB600125 decreased these responses. The blue arrow in the picture shows the implanted titanium particles and the red arrows show the immunohistochemical positive cells. **D**, *p<0.05 compared with the Sham group; n=5 per group.

Protein Expression for p-JNK by Western Blotting

The protein expression levels of p-JNK in the pouch membrane tissues were determined *via* western blot assays. The relative expression level of the Titanium group p-JNK protein was significantly higher than that of the Sham group (p<0.05), while the relative expression level of the Titanium + JNK inhibitor group p-JNK protein was significantly reduced compared with that of the Titanium group (p<0.05) (Figure 9).

Discussion

Periprosthetic osteolysis and consequent aseptic loosening is the most common reason for total joint arthroplasty failure and surgical revision. A significant factor in aseptic loosening following hip joint replacement is particle-induced osteolysis[11]. Through advancements in surgical technique, biomechanics, and prosthetic material design, numerous researchers have tried to lower aseptic loosening. These methods, however, are insufficient to reduce particle-induced osteolysis on their own since they are unable to stop particle formation from reaching the surfaces that support prosthetics[12]. In addition, current research has shown that cellular and molecular biological mechanisms contribute to the inflammatory osteolysis brought on by wear particles following total joint arthroplasty. These studies[11,12] identified the key roles that macrophages, osteoprogenitor cells, and osteoclasts and their secreted inflammatory and chemotactic mediators play in the process, as well as characterizing the different biological responses elicited by various wear particles caused by osteolysis[13,14]. It is worth mentioning that there is a co-participation of very low virulence microorganisms, most of which have never been identified, that survive on the metal surface in favorable biofilms and lead to secondary loosening of the implant[15]. Based on these views, sterile mobilization can also be caused

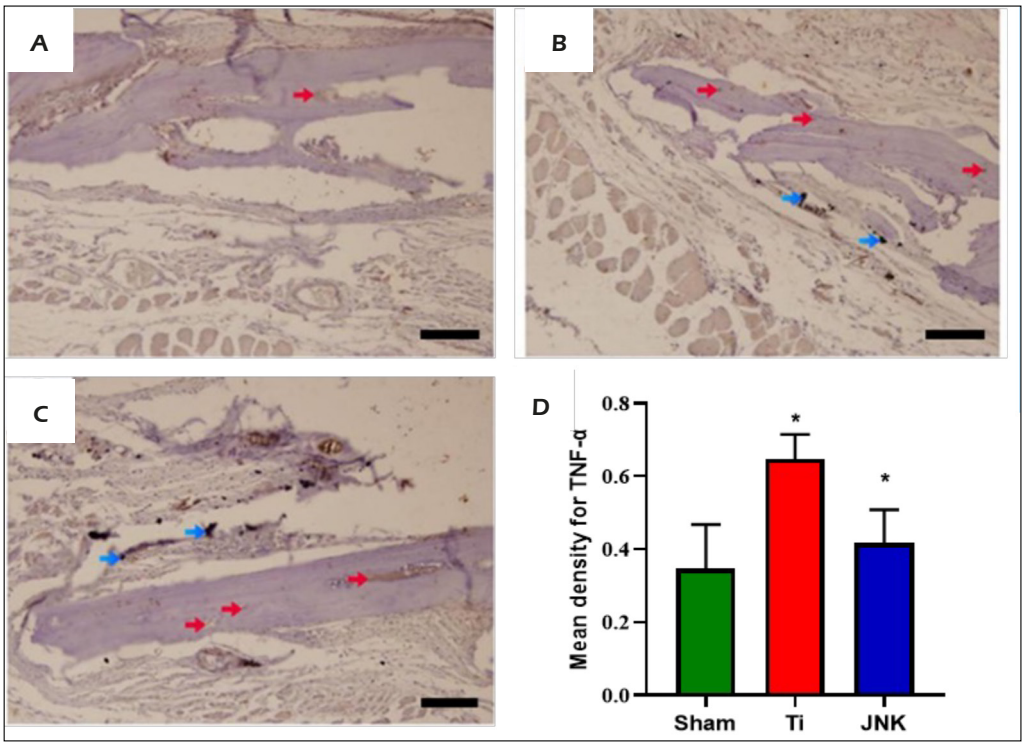

Figure 7. Immunohistochemical detection of TNF-α (×200). Air pouches in the Sham (**A**), Titanium (**B**), and Titanium + JNK inhibitor group (**C**). Titanium particles stimulated increased immunohistochemical positive cells, while SB600125 decreases these responses. The blue arrows in the picture show the implanted titanium particles, and the red arrows show the immunohistochemical positive cells. **D**, **p*<0.05 compared with the Sham group; n=5 per group.

by the complex coordination of multiple factors: bacteria, prosthesis, and host weakness. All of these studies demonstrated that molecular-biology-based techniques may be able to reverse the exacerbation of postoperative aseptic loosening of the artificial joints, at a non-invasive intervention level[16]. Few effective strategies, meanwhile, have been created to control this process brought on by wear particles. In an effort to provide a novel pharmaceutical approach to reduce wear particles-induced inflammatory osteolysis and consequent aseptic loosening, several therapies have been tested.

Aseptic loosening is thought to be primarily caused by inflammation and osteoclastogenesis stimulated by wear particles. TNF-α, IL-1, IL-6,

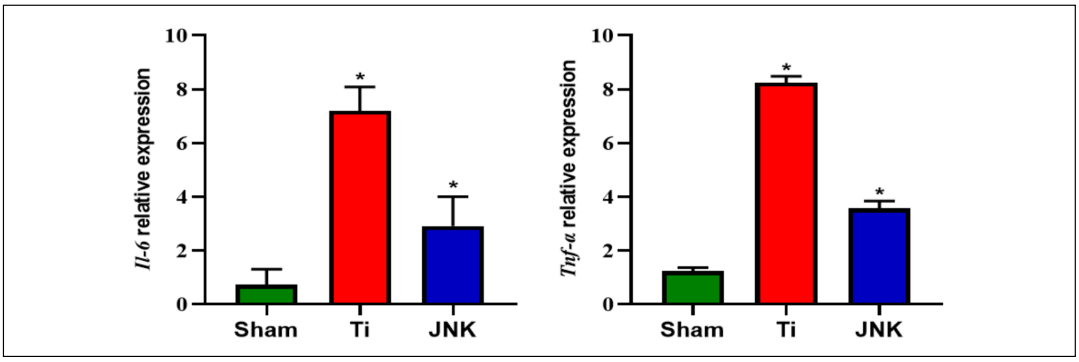

Figure 8. Comparison of the relative expression of IL-6 and TNF-α mRNA in three groups. (**p*<0.05 compared with the Sham group; n=5 per group).

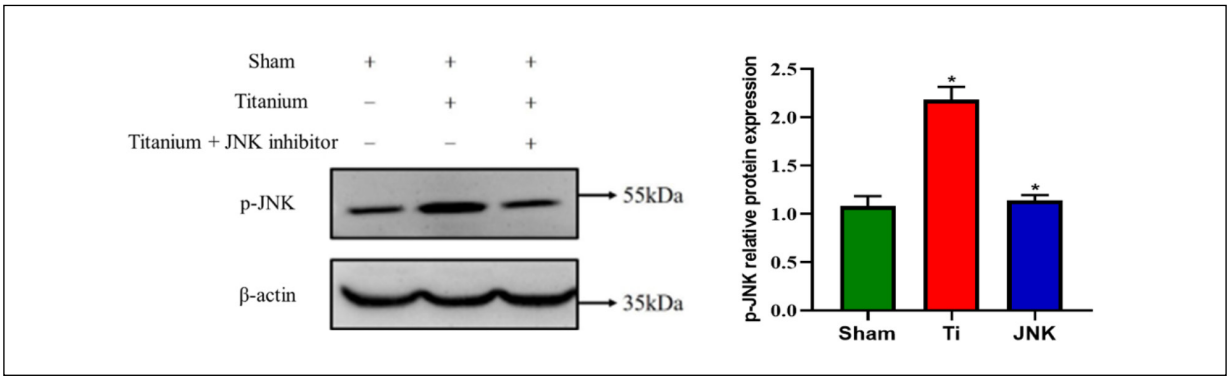

Figure 9. Relative expression results of three groups of p-JNK proteins. (*p<0.05 compared with the Sham group; n=5 per group)

IL-10, MMP-2, IL-6, and COX-2 are just a few of the inflammatory mediators and proteolytic factors that can be secreted by macrophages when they are stimulated by wear particles produced at the interface of a prosthesis and the peri-prosthetic tissue[17,18]. The findings of several studies[19] also imply that the JNK signaling pathway functions as important intracellular signal transducers in osteoclasts and osteoblasts and may be involved in the initiation and development of osteolysis. However, little literature has investigated how the JNK signaling pathway affects the inflammatory cytokines, chemokines, and enzymes involved in osteolysis when wear particles are stimulated. Consequently, the current study suggested that SB600125, a JNK inhibitor, may be a promising therapeutic strategy for preventing and treating wear particles-induced inflammatory osteolysis and subsequent aseptic loosening.

In this study, microscopic observation showed that the application of the JNK inhibitor (SP600125) could effectively reduce the inflammatory response caused by titanium particle stimulation, and the redness and swelling of the surrounding tissues were significantly reduced. H&E and TRAP staining showed that there were some inflammatory reactions around the surface of the skull with the titanium particles, the number of osteoclasts increased, a few clusters of macrophages and lymphocytes and other inflammatory cells infiltrated, there was more osteolysis, and the bone surface was not smooth. The above demonstrates titanium particles upregulate JNK activity in cartilage and synovial cells. This suggests that inflammatory cytokines may be important mediators of osteolysis-induced aseptic loosening. Compared with the Titanium group, in the JNK inhibitor group, inflammatory cells had significantly reduced infiltration and the thickness of the pouch membrane was significantly reduced, indicating that the JNK signaling pathway may be involved in the aseptic inflammatory response induced by the wear particle stimulation. Additionally, we found that treatment with SB600125, a particular JNK inhibitor, significantly reduced the TRAP-positive cells at the interface between the implanted skull and the pouch membrane in the Titanium group compared to the Sham group, implying that the JNK inhibitor could reduce wear particles-induced inflammatory osteolysis in air pouch osteolysis model. This further demonstrates the critical function the JNK signaling pathway plays in the inflammatory osteolysis brought on by wear particles.

Pro-inflammatory cytokines, such as TNF-α and IL-6, are known to be potent immunomodulators in activated macrophages and are also the key cytokines involved in periprosthetic osteolysis caused by wear particles. Cytokines and chemokines are important immune modulators released from immune cells in response to infection[20]. It was found in this study that the cytokine levels in the titanium particle stimulation group were significantly higher than those in the Sham group, and in the titanium + JNK inhibitor group, the relative expression of IL-6 and TNF-α was significantly reduced. RT-qPCR detected the levels of IL-6 and TNF-α mRNA, and the results were consistent with the results of immunohistochemistry. Inflammatory osteolysis caused by titanium particles leads to increased mRNA levels of IL-6 and TNF-α in osteoclasts in the skull and cyst wall of mice, and SB600125 inhibits this increase. These findings indicated that

JNK inhibitors could attenuate or block the JNK signaling pathway by competitively inhibiting the phosphorylation of JNK proteins. When the JNK signaling pathway is suppressed or blocked, the expression of IL-6 and TNF-α is reduced, thereby reducing the inflammatory response. The differentiation and activation of osteoclasts were alleviated, and finally, the occurrence of inflammatory osteolysis induced by abrasion particles was alleviated[21-23].

JNK signaling pathway has been used as a therapeutic target for disorders associated with inflammation, and it is a desirable pharmacological target due to its function in cytokine and enzyme-mediated regulation of inflammation. A recent study[24] discovered that the JNK signaling pathway may be effective in reducing the inflammatory osteolysis reaction because SCIO-323, a JNK inhibitor, regulates apoptosis and processes like osteoclast development at the level of gene transcription and mRNA translation. The results of our investigation lend credence to the notion that wear debris-induced inflammatory osteolysis may be alleviated by selective JNK inhibitor-mediated inhibition of cytokine production. In addition, recent studies have found that other signaling pathways related to MAPK and NF-κB can also influence titanium particle-induced bone resorption[25]. MAPKs are a class of serine/threonine protein kinases that are widely present in various biological cells, including mammals. They can be activated by various extracellular signals or stimuli, such as physical stress, inflammatory cytokines, growth factors, bacterial complexes, etc., and transmit them into the cell, thereby exerting biological activities. MAPKs play important roles in cellular signal transduction and regulation of cellular activities[26]. NF-κB is a crucial transcription factor that is involved in regulating cellular inflammatory responses, immune responses, cell proliferation, differentiation, and survival, among many other aspects[27]. Signal transduction pathways play significant roles in the occurrence of bone resorption. Chrysin protects against titanium particle-induced bone resorption by inhibiting NF-κB and MAPK signaling pathways, reducing osteoclast formation and function[28]. Lonafarnib inhibits Farnesyltransferase by suppressing the ERK signaling pathway, thus preventing osteoclast generation in titanium particle-induced bone resorption[29]. Rhoifolin improves titanium particle-stimulated bone resorption and reduces osteoclastogenesis through RANKL-induced NF-κB and MAPK pathways[30]. Carnosol inhibits RANKL-induced osteoclastogenesis and mitigates titanium particle-induced bone resorption[31]. Biochanin A exhibits inhibitory effects on osteoclast activation and inflammatory bone resorption induced by titanium particles through NF-κB and MAPK pathways[32]. Forsythiaside could inhibit RANKL-induced osteoclastogenesis and Ti particle-induced periprosthetic osteolysis *via* JNK, ERK, and p38 signaling pathways[33]. V-ATPase is a unique enzyme complex that distinguishes osteoclasts from macrophages and other cells[34]. These proton pumps play an important role in osteoclastic bone resorption by mediating extracellular acidification of the ruffled border membrane and bone surface in osteoclasts. Interestingly, titanium particle-induced bone resorption is related to V-ATPase activity in osteoclasts. The V-ATPase inhibitor saliphenylhalamide can attenuate wear particle-induced bone resorption in a mouse calvaria model by inhibiting NF-κB and ERK 1/2 signaling pathways during osteoclast differentiation[35]. We expect that further research will continue to investigate the effects of JNK inhibitor SB600125 in combination with these compounds on osteolysis, which would be a meaningful endeavor.

Conclusions

This research revealed that the JNK signaling pathway could be crucial for the development of osteolysis. By reducing JNK signaling pathway activity, the JNK inhibitor SB600125 can prevent the development of wear particle-induced inflammatory osteolysis. These results suggest that the JNK signaling pathway could be a useful therapeutic target for preventing and treating aseptic loosening caused by particle-induced inflammatory osteolysis.

Conflict of Interest

The authors declare that they have no conflict of interests.

Authors' Contribution

Zige Liu, Jiangbo Zhao, and Chen Zhang contributed equally to this work. JiangBo Zhao and Chen Zhang gathered the data. Zige Liu and Desheng Chen contributed to writing the discussion. Zige Liu and Yue Liu contributed to the experimental design. Zige Liu, Yulei Xie, Ang Yuan, and Wenpeng Wang wrote the paper. All authors approved the final version of the manuscript.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author upon reasonable request.

Ethics Approval

All animal-related experiments were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of NingXia Medical University (No.: 2015-019). All experiments were carried out under the standard principles of animal experiment ethics and complied with the Declaration of Helsinki.

Informed Consent

Not applicable.

Funding

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