Scutellarin inhibits RANKL-mediated osteoclastogenesis and titanium particle-induced osteolysis via suppression of NF-κB and MAPK signaling pathway

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ABSTRACT

Aseptic prosthetic loosening is a major complication after hip joint replacement. Wear particle-induced periprosthetic osteolysis plays a key role in aseptic prosthetic loosening. Attempting to modulate receptor activator of nuclear factor-κB (RANKL) mediated signaling pathways is a promising strategy to prevent aseptic prosthetic loosening. In the present study, we determined the effect of scutellarin (SCU) on titanium (Ti) particle-induced osteolysis in a mouse calvarial model and RANKL-mediated osteoclastogenesis. We determined that SCU, the major effective constituent of breviscapine isolated from a Chinese herb, has potential effects on preventing Ti particle-caused osteolysis in calvarial model of mouse. In vitro, SCU could suppress RANKL-mediated osteoclastogenesis, the function of osteoclast bone resorption, and the expression levels of osteoclast-specific genes (tartrate-resistant acid phosphatase (TRAP), cathepsin K, c-Fos, NFATc1). Further investigation indicated that SCU could inhibit RANKL-mediated MAPK and NF-κB signaling pathway, including JNK1/2, p38, ERK1/2, and IκBα phosphorylation. Taken together, these results indicate that SCU could inhibit osteoclastogenesis and prevent Ti particle-induced osteolysis by suppressing RANKL-mediated MAPK and NF-κB signaling pathway. These results suggest that SCU is a promising therapeutic agent for preventing wear particle-induced periprosthetic osteolysis.

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1. Introduction

Currently, joint replacement surgery is becoming an increasingly popular method to alleviate joint pain and improve patients' quality of life. However, aseptic prosthesis loosening has been a common complication of joint replacement surgeries and can dramatically shorten the service life of prostheses. Once prosthesis loosening occurs, it can eventually result in surgery failure and require revision surgery, which causes psychological and financial burden to patients [1,2]. Currently, the exact mechanisms mediating aseptic prostheses loosening are still unclear, but it is generally recognized that wear particles induce inflammatory responses that initiate periprosthetic osteolysis [3].

Many factors, such as biological and mechanical factors, lead to the production of different types of wear debris, such as metal and UHMWPE particles and cement debris, as well as others. These particles will recruit immune cells, such as macrophages and lymphocytes, to the surface of the prosthesis and bone [4]. After being activated by wear particles, they will release pro-inflammatory cytokines, such as TNF-α and IL-1β [5,6]. Subsequently, these pro-inflammatory cytokines will promote the expression of RANKL, which is an essential factor for osteoclastogenesis. After RANKL binds to receptor activator of nuclear factor-κB (RANK) [7], it leads to the activation of downstream signaling pathways, such as mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) [8], which play key roles in the process of osteoclast formation and bone absorption function.

Scutellarin (SCU), a novel flavonoid agent, is the major effective component of a Chinese herb breviscapine [9,10]. In the clinic, scutellarin was found to be effective when used for treating some diseases, such as ischemic cardiovascular and cerebrovascular diseases [11,12]. It is reported that SCU has many pharmacological features, such as anti-proliferation and anti-inflammation [13]. Moreover, SCU can decrease caspase-3 activity and inhibit p38 phosphorylation in PC12 cells [10]. Also, SCU can also protect lipopolysaccharide (LPS)-

induced acute lung injury in mice by inhibiting the NF-kB signaling pathway [14], and it down-regulates MUC5AC mucin production on HBE16 cells by suppressing the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) [15]. However, whether it has an effect on osteoclastogenesis remains unknown.

Therefore, in this study, we aimed to determine the effects of SCU on osteoclastogenesis and wear particle-induced osteolysis and investigated the underlying mechanisms. The findings of this study may provide a new method for preventing wear particle-induced prosthesis loosening in the clinic.

2. Materials and methods

2.1. Cells, media and reagents

The Raw264.7 cell line was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Bone marrow-derived macrophages (BMMs) were harvested from 4-week-old C57BL/6 male mice, as previously described [16,17]. SCU was purchased from the Institute of Traditional Chinese Medicine of Shanghai (Shanghai, China). The Cell Counting Kit was purchased from Dojindo Molecular Technology (Japan). M-CSF (recombinant soluble mouse) and RANKL were purchased from Peprotech (USA). The tartrate-resistant acid phosphatase (TRAP) staining kit was purchased from Sigma Aldrich (St Louis, MO, USA). Specific antibodies against phospho-IκBα, ERK1/2, phospho-ERK1/2, JNK, phospho-JNK, p38, phospho-p38, c-Fos, and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) were purchased from Cell Signaling Technology (Cambridge, MA, USA).

2.2. Cell preparation

BMMs were harvested from 4 week-old C57BL/6 male mice and cultured in α-minimum essential medium (α-MEM) and 10 ng/mL M-CSF for 24 h. Next, the adherent cells were collected and cultured in a 37 °C, 5% CO_2 incubator for another 3 to 4 days. Once the cells reached approximately 80% confluence, they were used for experimentation.

2.3. Cell viability

The Cell Counting Kit assay was applied to exclude the influence of SCU on viability of BMMs and Raw264.7 cells. Briefly, BMMs and RAW264.7 cells were cultured in a 96-well plate and stimulated with M-CSF (30 ng/mL) and RANKL (60 ng/mL), and subsequently treated with the indicated doses of SCU for 48 h. Next, 10 μL of CCK-8 assay solution was added to each well, and the cells were incubated for an additional 2 h. The absorbance values of each well were measured using an enzyme standard instrument, and the following formula was used to test the cell viability: [experimental group OD-zeroing OD]/[control group OD-zeroing OD].

2.4. TRAP staining

TRAP staining was preformed to examine the inhibiting effects of SCU on osteoclast differentiation. BMMs were plated into a 6-well-plate at a density of 1.2×10^5 cells/well and different concentrations of SCU (0, 2.5, 5 or 10 μM), as well as M-CSF (30 ng/mL) and RANKL (60 ng/mL), were added. Every 2 days, the culture medium was replaced until mature osteoclasts were formed on day 7. Subsequently, the original medium was removed, and 4% (w/v) paraformaldehyde
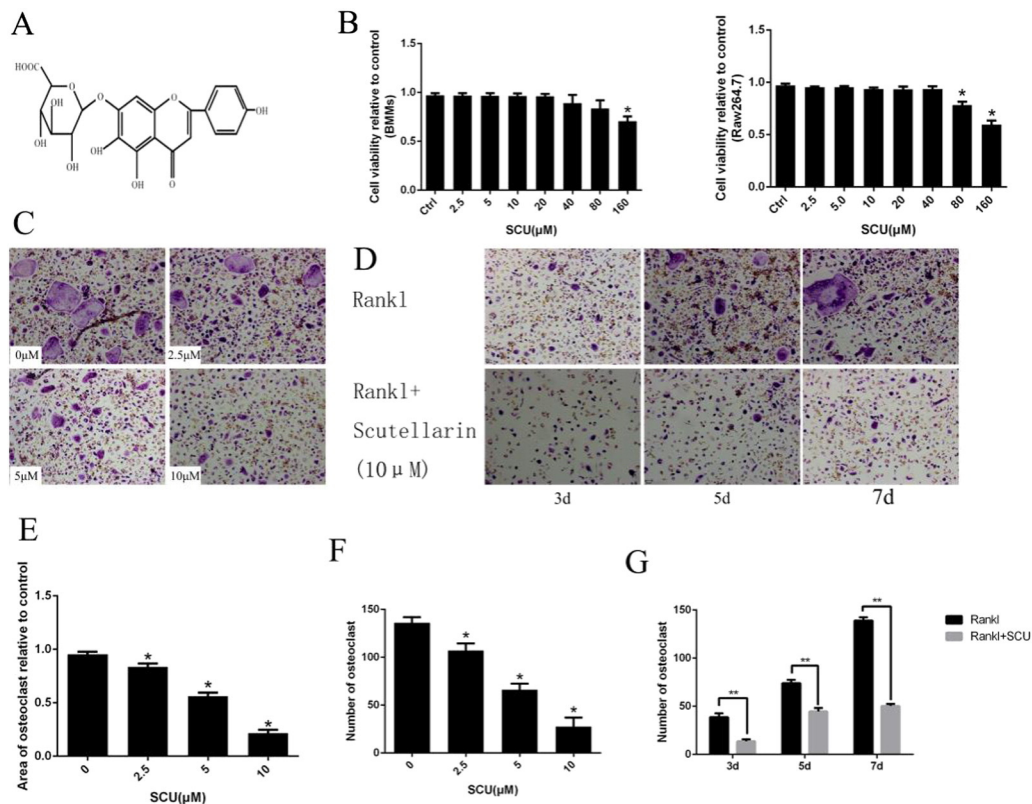

Fig. 1. Scutellarin (SCU) reduced RANKL-mediated osteoclast formation without influences on viability of BMMs or Raw264.7 cells. (A) Chemical structure of Scutellarin. (B) Cells were stimulated with 30 ng/mL M-CSF and 60 ng/mL RANKL, and different concentrations of scutellarin was added for 48 h and then a CCK-8 assay was used to measure cell viability. (C) BMMs were stimulated with 30 ng/mL M-CSF and 60 ng/mL RANKL and treated with the indicated concentrations of scutellarin for 7 days. 4% (w/v) paraformaldehyde fixed and performed TRAP staining. (D) BMMs were treated with or without 10 μM scutellarin and stimulated with 30 ng/mL M-CSF and 60 ng/mL Rancor 3, 5 and 7 days, respectively and performed TRAP staining. (E)The area of osteoclasts relative to control (*p < 0.05 versus the control). (F) The counting of TRAP-positive osteoclasts (*p < 0.05 versus the control). (G) The counting of TRAP-positive osteoclasts at 3, 5 and 7 days. (**p < 0.01).