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The potential role of strontium ranelate in treating particle-induced osteolysis

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ABSTRACT

Ultra high molecular weight polyethylene (UHMWPE) wear-particle-induced osteolysis is one of the major issues affecting the long-term survival of total joint prostheses. Currently, there are no effective therapeutic options to prevent osteolysis from occurring. The aim of this study was to evaluate the role of strontium ranelate (SR) in reducing the risk of particle-induced osteolysis. Forty-eight C57BL/6J ultra-high molecular weight polyethylene (UHMWPE) particle-induced murine calvarial osteolysis models were used. The mice were randomized into four groups as: sham (Group 1), UHMWPE particles (Group 2), and SR with UHMWPE particles (Group 3 and Group 4). Groups 1 to 3 were sacrificed at two weeks and group 4 was sacrificed at the fourth week. The skulls were then analyzed with a high-resolution micro-CT. Histological evaluation was then conducted and osteoclast numbers were analyzed for comparison. Based on the micro-CT, percentage bone volume and trabecular thickness were found to be significantly higher in Group 4 than in Group 2 ($p < 0.001$). Osteoclast numbers in SR treated groups (Group 3 and Group 4) were reduced when compared to groups that did not receive SR treatment (Group 2). These results indicated that SR treatment helps to increase bone volume percentage and trabecular thickness and also suppresses osteoclast proliferation. It is suggested that oral SR treatment could serve as an alternative therapy for preventing particle-induced osteolysis.

1. Introduction

Artificial joint replacement is considered an effective method for treating severe joint degeneration. However, periprosthetic osteolysis resulting from the generation of ultra high molecular weight polyethylene (UHMWPE) wear particles in artificial joints is one of the major long-term complications following total joint arthroplasty [1,2]. The wear particles stimulate inflammatory responses and osteoclastic resorption processes at the bone-implant interface, which consequently leads to implant loosening.

Previous studies have focused on identifying drugs which may reduce the incidence or severity of osteolysis [2–4]. Bisphosphonates (BPs) have been proven to be effective in preventing metabolic bone loss conditions such as osteoporosis and Paget's disease. BPs are especially effective in reducing the risk of hip and spine fractures [5]. It has also been hypothesized that BPs could inhibit particle-induced osteolysis in *vivo* [6–8]. However, adversely, oral BPs may increase the risk of osteomyelitis of the jaw when compared to patients treated with other osteoporosis medications [9]. Side effects of long-term BP treatment may also include osteonecrosis of the jaw, atypical femur fractures, atrial fibrillation, and esophageal cancer [10]. As a result, the U.S. Food & Drug Administration (FDA) issued an alert highlighting the possibility of severe and sometimes incapacitating bone, joint and/or muscle pain in patients taking BPs.

In addition to BPs, strontium ranelate (SR) has been approved as an alternative medicine for treating postmenopausal osteoporosis. The advantages of SR as an alternative medicine are that SR

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stimulates pre-osteoblastic cell replication, suppresses osteoclastic differentiation and also increases osteoclastic apoptosis [11]. As a result, SR treatment has been shown to reduce bone loss [12,13], improve trabecular and cortical architecture [14], and increase mechanical fixation of implants [15]. Wear debris-induced osteolysis is still a major complication affecting the long-term success of joint replacements. Drugs used for treating osteoporosis, such as BPs, have been proven to hinder the osteolytic process [16,17]. However, the associated side effects are still significant issues. This raises an uncertainty regarding whether or not SRs could potentially serve as an alternative therapeutic option for particle-induced osteolysis. To our knowledge, there are relatively few studies that have tested the effects of oral SR in treating osteolysis. Thus, the current study attempts to investigate the effects of oral SR on UHMWPE wear particle-induced osteolysis by using a well-established murine calvarial model. It was hypothesized that the SR treatment could reduce the risk of particle-induced osteolysis.

2. Materials and methods

2.1. Polyethylene particle preparation

Conventional ultra high molecular weight polyethylene (UHMWPE, GUR 1020) was purchased from Orthoplastics Ltd. (Lancashire, United Kingdom). Submicron-sized particles were produced by our coauthor according to published methods [18]. The particles were further characterized using light scattering analysis [19] and scanning electron microscopy to confirm their size and shape distribution. The mean particle size was determined from SEM images whereby one hundred particles were randomly selected to measure the particle size and aspect ratio. The mean size and the aseptic ratio of the particles were 0.77 ± 0.34 μm and 1.85 ± 0.83, respectively (Fig. 1). Endotoxin detection was then used to ensure that the endotoxin content was below 0.06 EU/ml, in compliance with animal testing standards (according to FDA published guidelines for LAL testing) [20]. The experimenter dispersed the wear particles in hyaluronic acid (HA) to form the wear particle suspension liquid.

2.2. Animals and surgical treatment

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee where the study was performed. Forty-eight eighteen-week old female C57BL/6J mice, weighing 21–25 g, were purchased from BioLASCO, Taipei, Taiwan, an AAALAC international certified biotechnology company. The animals were kept in a room at 24 °C, 50% humidity, and with a 12 h light/dark cycle (light from AM 7:00 to PM 7:00).

The *in vivo* calvaria experiments were performed with reference to similar previous experiments [6,21,22]. The mice were briefly anesthetized with 100 mg/kg of ketamine and 10 mg/kg of rompun by intraperitoneal (i.p.) injection. A 0.5×0.5 cm area of calvarial bone was then exposed by making a midline sagittal incision over the calvaria, leaving the periosteum intact. UHMWPE particles were spread over the area and the incision was closed with sutures. In Group 1 ($n = 12$), the mice were not injected with UHMWPE particles and acted as the sham group. Group 2 ($n = 12$) mice were injected with 1 mg of wear particles in the calvarial bone and received 0.5% carboxymethylcellulose aqueous solution by gavaging with volumes corresponding to those administered in the SR-treated groups (Group 3 and Group 4). In Group 3 ($n = 12$) and Group 4 ($n = 12$), the same mass of 1 mg of wear particles was injected and the mice were orally treated with strontium ranelate (S12911-2, PROTELOS®, Servier, France) by gavaging a dose of 1800 mg/day/kg [14], 5 days/week. This dosage creates a blood strontium concentration close to the equivalent human concentration after a therapeutic dose of 2 g/day [14]. Groups 1, 2 and 3 were sacrificed after 2 weeks and Group 4 was sacrificed after 4 weeks.

2.3. Micro CT imaging analysis

The cranium was fixed in 10% buffered formalin for 24 h, and then transferred to 70% ethanol for 24 h, before being analyzed by a Skyscan 1076 (Bruker micro-CT, Kontich, Belgium). The micro-CT was then set in a 2048×2048 pixel matrix, and three-dimensional images were reconstructed with a voxel size of 18 μm. The micro-CT analysis primarily focused on the osseous properties of the sagittal suture [21]. To minimize bias from the 3D orientation of the calvaria, a spherical volume of interest (VOI) of 4 mm in diameter was defined with the midline suture of the skull at its center (Fig. 2). For quantitative analysis of polyethylene particle-induced osteolysis, the resident software was used to obtain the following parameters within the VOI: bone volume (BV), tissue volume (TV) and trabecular thickness ($Tb \cdot Th$). To quantitatively assess the particle induced bone loss, the results were expressed as the mean difference of BV/TV [Δ(BV/TV)] and mean difference of $Tb \cdot Th$ [$\Delta(Tb \cdot Th)$] between sham-operated animals and particle-treated animals.

2.4. Histological evaluation of osteolysis and osteoclast numbers

After micro-CT scanning, calvaria were decalcificated, dehydrated, and embedded in paraffin sections. At intervals of 400 μm, coronal (transverse) sections (5 μm thick) were made through each specimen using a microtome (KeDi Instruments Equipment Co., Zhejiang, China), producing three sections of the particle-treated area per sample, and which were consistently located over the sagittal suture. Sections were stained with Hematoxylin and Eosin (H&E stain) to observe the periprosthetic membrane of the implantation and to observe if factors related to pre-inflammation in osteolysis were present, including the presence of multinucleated giant cells. In addition, a polarized light microscope was used to observe the wear particle distribution within the tissue. Also, a tartrate resistant acid phosphatase (TRAP) stain was used to observe if the osteoclast numbers were undergoing abnormal activation. The number of osteoclasts was then determined by counting the number or TRAP-positive multinucleated cells by two coauthors (YCL, TKC) that were blinded to the data.

2.5. Statistical analysis

In order to determine the total sample size, power analysis was used based on our previous experience when analyzing the BMD. It was found that having 11 samples in each group could achieve 90% power. The estimated means were 0.55, 0.40, 0.45, and 0.45, and the estimated standard deviation and effect size were 0.09 and 0.61, respectively. The current study used 48 mice in total and could achieve 90% power at a significance level of 0.05.

Data were reported as mean ± SD. A two-sided Student's t-test was performed for independent samples to analyze the difference in means between groups. The results were analyzed by analysis of variance (ANOVA) to show the difference between groups. Multiple comparisons were adjusted with a Bonferroni post hoc test. p-values less than 0.05 were considered significantly different.