ERYTHROMYCIN INHIBITS RANK/RANKL SIGNALING AND INFLAMMATORY OSTEOLYSIS INDUCED BY UHMWPE DEBRIS IN A MURINE MODEL.

INTRODUCTION
Aseptic loosening due to wear debris-induced osteolysis remains a major clinical problem, and is the most prevalent complication of total joint arthroplasty. Activation of RANK (receptor activator of nuclear factor kappa B)/RANKL (RANK ligand) signaling and inflammatory osteolysis in response to orthopaedic wear debris may be critical in the development of aseptic loosening. Erythromycin (EM), a macrolide antibiotic, has been reported to be effective in improving chronic inflammation and resultant tissue injury by inhibiting NFkB (nuclear factor kappa B) activity. The purpose of this study was to examine whether EM can suppress wear debris induced inflammatory osteolysis in our recent developed murine model of wear debris-induced osteolysis.

MATERIALS & METHODS
Murine model of wear debris-induced osteolysis Air pouches were established by the subcutaneous injection of sterile air on the back of BALB/c mice, followed by introduction of 0.5mg of UHMWPE debris suspended in saline. Two days later, a section of calvaria from a syngeneic mouse was inserted into the pouch. EM dissolved in 0.9% saline was given to mice intraperitoneally at a dose of 2 mg/kg/d from day 1 to day 14 after UHMWPE inoculation. Both EM untreated mice and mice injected with saline alone (no UHMWPE) were included as controls. Tissues were harvested 14 days after bone implantation for morphological and molecular analysis. Institutional approval was obtained for all animal experiments.

Real time RT-PCR assay Gene level of RANK, RANKL, IL-1β, TNFα and Cathespin K (CK) was determined by real-time RT-PCR technique. GADPH transcripts were used as an internal control. The number of target gene copies was calculated by regression analysis against the standard curve or controls.

Histology assessment Paraffin tissue sections were stained with Hematoxylin & Eosin (H&E) for morphology analysis. Van Gieson stain was used to detect collagen. Osteoclast activity was determined by tartrate-resistant acid phosphatase (TRAP) staining in frozen tissue sections. Digital histological images were analyzed by a computerized image analysis system.

Statistical analysis Mice were randomly assigned to three experiment groups with 8 animals in each group. Statistical analysis between groups was performed by the two-tailed paired-samples T-test. The Kruskal-Wallis test was used to compare the differences for more than two groups. A p value of less than 0.05 was considered as significant difference.

RESULTS
Image analysis of H&E stained histology sections showed that both the thickness (0.099±0.02 mm) and the cellular infiltration (5990±1033/mm²) of the pouch membrane in EM treated mice were significantly decreased in comparison with untreated mice (0.14±0.04 mm and 7888±1524/mm² respectively, p<0.05). Real time RT-PCR assay indicated that gene expression of IL-1β (p<0.05) and TNFα (p<0.01) in tissue from pouches of EM treated mice was significantly lower than in tissues of untreated mice. Interestingly, EM treatment also resulted in a significant reduction of both RANK (1.33E+05 ± 3.36E+04, p<0.05) and RANKL (2.27E+03 ± 1.45E+03, p<0.01) gene levels in the model, compared with untreated mice (RANK, 2.48E+05 ± 7.08E+04; RANKL, 9.50E+03 ± 5.54E+02) and saline controls, and the osteoclast marker Cathespin K mRNA level was also decreased by EM treatment (Figure 1, p<0.05). Osteoclast activity was determined by TRAP staining on frozen tissue sections. In untreated mice, a large number of TRAP⁺ cells were found both in the implanted calvaria, and at its contact site with inflammatory tissue, where high levels of active osteolysis were observed. However, treatment with EM caused a significant reduction in the number of TRAP⁺ cells (Figure 2). Consistent with the TRAP staining, Van Gilson staining revealed that EM treatment significantly inhibited collagen loss from calvaria in close contact with inflammatory membranes (15.1% loss), in comparison with similar regions in untreated mice (36.6% loss, p<0.05).

Figure 1. EM suppresses UHMWPE- induced over gene expression of Cathespin K

Figure 2. EM inhibits UHMWPE- induced inflammatory osteoclastogenesis as shown by histochemical TRAP staining on tissue frozen section.

DISCUSSION
The interactive network of bone-resorbing cytokines converges at the level of RANK/RANKL pathway that might serve as the final common mediator of osteoclastogenesis. Activation of RANK/RANKL pathway may be critical in the development of aseptic loosening. In this study we have provided the first evidence that EM suppresses wear debris-induced inflammatory osteolysis by down-regulation of RANK/RANKL signaling pathway. Potential reasons for the use of EM in bone loss modification include its excellent long-term side effect profile and its property of concentrating in bone and inflammatory cells, especially in macrophages, the reservoir of osteoclast precursors. Therefore, EM might represent a promising therapeutic candidate to treat or prevent wear debris-associated aseptic loosening.

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REFERENCE