Local erythromycin delivery in a rat osteolysis model

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INTRODUCTION

We have demonstrated that erythromycin (EM) inhibits wear debris- induced macrophage activation and osteoclastogenesis (both *in vitro* and *in vivo*) through targeting NF-κB signaling [1,2]. Our clinical trial further verified that oral EM can be efficiently delivered to periprosthetic tissue and improve local inflammation. The purpose of this study was to assess the efficacy of local EM delivery on particle- induced osteolysis in a rat model.

MATERIALS AND METHODS

Loading of EM solution on the *PA-coated Ti pin*

The Ti pin (diameter 1 mm, length: 10 mm, with a flat head of 1.2 mm diameter) was coated with Peri-Apatite™ (PA) (Stryker, thickness 30-50 μm). EM (SIGMA) solution (350 mg/ml in sterile PBS/ethanol) was applied to the PA layer of the Ti pin (8 μl = 2.8 mg/pin). Zone of *S. Aureu* inhibition assay showed around 25% of loaded EM was remained in the PA layer 24 hours after loading.

Rat osteolysis model (Table)

Group	n	Description
I	5	Pin + Saline (control)
II	7	Pin + UHMWPE
III	7	EM-coated pin + UHMWPE

Nineteen (19) S.D. rats (6 months old) were divided into 3 groups (Table). Uncoated Ti pins were press- fit inserted into right tibia following the injection 200 μl of either UHMWPE particles (mean size of 2.6 μm, 5 mg/ml) or saline (control). The revision surgeries were performed 8 weeks after the first surgery (development of loosening knee joints after UHMWPE stimulation). The previous implanted pins were replaced with new Ti pins either with or without EM coating. Rats were then sacrificed one month after "revision surgery", and the knee joint samples were collected for μCT and histology analysis.

μCT measurement Knee joint specimen were fixed vertically within the sample holder and placed in the uCT (SCANCO uCT 80; Switzerland) chamber. Specimens were scanned at energy of 70 kV and an intensity of 114 μA (resolution 20 μm). Integration time was set at 800 ms. A threshold was set to distinguish the bone and the void. The cortical bone was excluded from the region of interest, which covered a length of 260 μm distal to the tip of the pin. Structural indices were assessed with 3-D techniques.

Histology analysis Transverse sections of each tibia (2 mm from the distal end of Ti pin) were stained with H&E for tissue response and toluidine blue for connective tissue matrix content. Osteoclast formation was determined by TRAP staining. The thickness of periprosthetic membrane was measured at four different regions of H&E stained sections.

Statistical analysis was performed using the ANOVA method.

RESULTS

*μCT measurement (*Fig. 1)

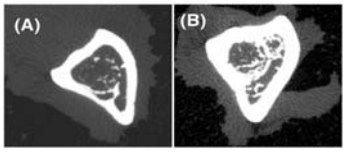

Fig. 1. μCT images of rat tibia (A) untreated, and (B) EM-treated.

Bone volume percentage was measured in the region and the value of the group treated with EM (Group III) (0.26 ± 0,07) was significantly higher than the group untreated (Group II) (0.14 ± 0.04), while there was no significant difference between Group III and the saline control (Group I)(0.15 ± 0.11). The parameters of cancellous bone structure all pointed a trend of better structure in Group III than that of Group I and II, i.e., a higher connectivity density (19.32 vs. 11.17 and 13.88), higher number of trabecular bone (2.02 vs. 1.57 and 1.72), a thicker of trabecular bone (0.16 vs. 0.13 and 0.12) and less space between the trabecular bone (0.52 vs. 0.76 and 0.64). However, this difference did not reach statistical significance.

Histology Images of H&E sections showed that in the saline control (Group 1) the tibia retained a smooth endocortical surface with a prominent periprosthetic membrane. In the EM- treated group, endocortical erosion was reduced and the periprosthetic tissue appeared thinner than uncoated pins. The overall cellularity of periprosthetic membranes from the EM-treated group was decreased compared to the untreated group. Analysis of membrane thickness revealed a significantly thinner membrane in EM-treated group compared with untreated group and saline control ($p<0.05$). Increased density of TRAP staining was observed in periprosthetic interface with after UHMWPE stimulation, while osteoclast numbers were reduced by EM treatment.

DISCUSSION

A major limitation in the treatment of periprosthetic osteolysis is the lack of an effective local drug delivery system. Using a rat revision arthroplasty model, data indicated that an EM coated Ti pin provided a sufficient drug source to effectively treat wear debris- induced periprosthetic inflammation and osteolysis. Accordingly, the local EM delivery through Ti pin coating may be useful in preventing and treating particle- induced osteolysis around total joint replacement.

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REFERENCES

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