Mechanical Perturbations and Metal Particles Exaggerate Bone Resorption and Inflammation in MC3T3E1 Cells

INTRODUCTION

Metal implants are commonly used for spine, hip and knee reconstructive surgeries. Total hip arthroplasty is an effective surgical therapy for the replacement of defective joints and has been increasing in United States. Metal wear particle reaction, however, remains a major problem which limits the long-term success of implants. (1) Many studies have independently shown that wear debris from bone implants and mechanical perturbation from dynamic joint movement induces inflammatory osteolysis, resulting in aseptic loosening in total joint implants and spine wound drainage. Little is known, however, regarding the synergetic effect of mechanical perturbation and titanium (Ti) particles on osteolysis. The objective of this study was to investigate whether mechanical strain can exaggerate osteolysis induced by titanium particles at normal and super physiological levels.

METHODS

Cell Culture

MC3T3E1 cells were purchased from American Type Culture Collection (ATCC; Manassas, MD, USA) and maintained in Minimum Essential Medium Alpha (MEM) with 10% fetal bovine serum (FBS; Gemini BIO) and 1% antibiotic/antimycotic (Gemini BIO).

Particle Treatment

Since the most common sizes of particle from implants were 1-10um, pure titanium particles with an average diameter less than 10um, were purchased from Alfa Aesar. (2) For removal of LPS, the particles were incubated in 70% ethanol for 24hr and sterilized. The endotoxin level was determined by Limulus Amebocyte Lysate kit (i.e.<0.1EU/ml). Prior to use, particles were sonicated for 15 minutes. MC3T3E1 cells were incubated with particles of 0.47mg/cm^2 density for 3hr.

Mechanical Cyclic Strain

2 x 10^3 MC3T3E1 cells were seeded on 6-well tissue culture plates with a silicone elastomer membrane (Flexcell) and were incubated for 2 days. Cells were exposed to physiologic stretch (0.5%) and super physiologic stretch (5%) at 1Hz for 3hr.

Cell Transfection

For transfection, 1 x 10^5 MC3T3E1 cells were seeded on 6-well tissue culture plates (Flexcell) overnight and transfected with 2 µg of cDNA using the FuGene HD transfection reagent (Roche) according to the manufacturer’s instructions. After particle treatment, unpigocyotised particle were removed by washing with PBS. Phagocytosis was determined by measuring the area percentage phagocytosed by particles via customized program.

RNA Isolation and Real-Time RT-PCR

Immediately after end of stretch, RNA was extracted for gene expression analysis. Cells were lysated and total RNA isolated using RNeasy Mini Kit (Qiagen). The 260/280 absorbance ratio was measured for verification of the purity of RNA. The extracted RNA was used for cDNA synthesis by reverse transcriptase using SuperScript III system (Invitrogen). Analysis by quantitative real-time RT-PCR (Eppendorf) was conducted to determine the mRNA levels of MCSF, COX2 and the housekeeping gene GAPDH (Invitrogen). Each RNA sample was analyzed in triplicate. For each sample, mRNA levels of each gene were normalized to GAPDH levels.

RESULTS

Phagocytosis of particles was verified using the cell transfection method described above. With a titanium particle density of 0.47mg/cm^2, phagocytosed particles covered an average 21% of MC3T3E1 cell surfaces (n=5) (Fig1).

In MCSF mRNA experiments (n=4), MC3T3E1 cells with only particle treatment significantly increased by 160% compared to the control group. The physiologic stretch group did not statistically change while the super physiologic stretch group increased by 220%. The combined groups of physiologic stretch with particles and super physiologic stretch with particles resulted in a synergistic increase of 240% and 340% respectively. (Fig 2)

In COX2 mRNA experiments (n=4), there were significant increases in particle (410%), physiologic (160%) and super physiologic groups (220%). Synergistic increases were also seen in the combined groups of physiologic stretch with particles (446%) and super physiologic stretch with particles (594%). (Fig2)

DISCUSSION

The question addressed by this study was whether cyclic strain affects the way MC3T3 cells respond to titanium particles. The main finding of this study is that mechanical stretch could exaggerate the osteolysis induced by titanium particles. As a bone resorption stimulator, MCSF mRNA increased in the super physiologic strain with particles group. Particles appeared to be less effective for bone resorption and inflammation without mechanical stimulation. On the other hand, depending on magnitude of mechanical loading, more osteolysis could be induced. These results may imply that possible bone loss due to wear debris could be accelerated by severe physical loading.

REFERENCES


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