

Wear Particulates Protect Osteoclasts from Apoptosis Induced by Mechanical Strain *in vitro*.

Chad P. Coles, Robert W. Gilbert, Ying Fang Chen and Gail I. Anderson.

Depts of Surgery and Biomedical Engineering, Dalhousie University, Halifax, Canada.

INTRODUCTION: Bone responds to dynamic loading through effects on both osteoblastic bone formation and osteoclastic resorption¹. The mechanism by which mechanical loading down regulates osteoclast activity is not well understood. One potential control pathway is by programmed cell death, or apoptosis. Osteoclastic resorption also plays a role in aseptic loosening of arthroplasty components, which is believed to be promoted by wear particles² or fluid pressure³ or a combination of both these factors. We investigated the effects of mechanical strain on osteoclast apoptosis *in vitro*, as well as the effect of arthroplasty wear particles on osteoclast survival with and without mechanical stimulation.

METHODS: Osteoclasts were harvested from the long bones of neonatal NZW rabbits, and cultured on flexible-bottomed, collagen 1 coated dishes (Bioflex) in α -MEM with 15% FCS, vitamins C and D₃, and antibiotics. Dynamic biaxial mechanical strain (5000 microstrain), was applied cyclically during culture days 2 to 4 at frequencies of 1, 0.66, 0.33, and 0.166 Hz. Apoptosis in osteoclasts was suggested by morphological changes and confirmed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining. Cells were also cultured and strained in the presence of arthroplasty wear particles (polyethylene, polymethylmethacrylate, titanium, and cobalt chrome). High molecular weight polyethylene (PE) particulates were generated by a hip simulator⁴. The polymethylmethacrylate (PMMA) used was commercially-available Simplex powder. Commercially-available titanium particulates (Ti) were sieved to yield particulates with a mean diameter less than 5 microns. Cobalt chrome (CoCr) was from metal on metal tribology experiments using Sulzer components and was a gift from Dr John Medley, University of Waterloo, Ontario, Canada. The effects of inhibition of eicosanoids in this system were also investigated using indomethacin (IND) as a mixed COX-1 and COX-2 inhibitor and meloxicam (MET) as a COX-2 inhibitor. The effect of

inhibition of 5-lipoxygenase, and thus leukotriene synthesis, was also investigated using ICI 230487.

RESULTS: In control conditions (non-strained cells) 8.2% of osteoclasts demonstrated hallmarks of apoptosis. In response to mechanical strain the percentage of apoptotic cells was significantly increased to 22%, 21%, 17%, and 16% when exposed to 5000 μ strain at rates of 1, 0.66, 0.33 and 0.16 Hz, respectively ($p < 0.05$). Stromal cells (osteoblastic) did not demonstrate hallmarks of apoptosis in either mechanically-strained or non-strained conditions. The presence of wear particles significantly decreased the incidence of apoptosis in mechanically - stimulated osteoclasts. Ti and CoCr however appeared to induce apoptosis in the absence of mechanical stimulation whereas PE and PMMA did not ($p < 0.05$). The presence of IND and MET spared the osteoclasts from the mechanically-induced apoptosis. The 5-lipoxygenase inhibitor results were equivocal.

DISCUSSION & CONCLUSIONS: Mechanical stimulation of osteoclasts at physiologic levels induces apoptosis. This is a novel discovery, not previously reported. Arthroplasty wear particles have a protective effect, decreasing the rate of apoptosis. It appears that there is a role for eicosanoids in this process and that inhibition of cyclo-oxygenase mediated prostaglandin production protects against the induction of mechanically stimulated osteoclast apoptosis. The role of leukotrienes in this system is less clear. Particle-mediated circumvention of the physiological role of dynamic strain may help to explain the increased osteolysis associated with aseptic loosening.

REFERENCES: ¹DJ Mason et al. (1997) *Bone* 20 (3) 199-205. ²GI Anderson et al (2001) *J Biomed Materials Research – Applied Biomaterials* – in press. ³R Skripitz & P Aspenberg P (2000) *J Ortho Res.* 18 (3) 481-484. ⁴A Essner et al. (1996) *Biomaterials* 17: 865-871.

ACKNOWLEDGEMENTS: This work was supported by an operating grant from The Arthritis Society of Canada and a Clinical Research

Fellowship from the Dept of Surgery, Dalhousie University.