Polymethylmethacrylate Particles Decrease Osteoprogenitor Viability by Cell Necrosis Not Apoptosis

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INTRODUCTION:
Polymethylmethacrylate (PMMA) particles have been previously shown to inhibit the osteogenic differentiation of MC3T3-E1 osteoprogenitor cells with respect to mineralization and alkaline phosphatase expression. However, the mechanism of this inhibitory effect has not been studied. The inhibition of osteoprogenitor differentiation by PMMA particles may involve impairment of cell viability through necrosis or apoptosis. The purpose of this study was to determine whether PMMA particles are cytotoxic towards MC3T3-E1 osteoprogenitor cells and to determine whether cell death occurs by necrosis or apoptosis.

METHODS:
MC3T3-E1 subclone 14 osteoprogenitor cells (American Type Culture Collection) in 12-well plates were grown to confluency in ascorbic acid-free α-MEM with 10% FBS. After the first medium replacement, the cells were challenged with PMMA particles (1-10 μm, Polysciences) at concentrations of 0.000, 0.038, 0.075, 0.150, 0.300, and 0.600% v/v in 1.0 ml/well of medium for 72 hrs. Cell death was assessed by measuring levels of lactate dehydrogenase (LDH), an intracellular enzyme released from dead cells, in culture supernatant at 24, 48, and 72 hrs using the Cytotox96 Non-Radioactive Cytotoxicity Assay (Promega). The number of adherent viable cells was determined each day by hemocytometer cell count with trypan blue staining. A TUNEL assay was performed using the APO-BrdU Apoptosis Detection Kit (eBioscience) for detecting cells with fragmented DNA, a hallmark of apoptosis. To determine particle effects on proliferation, MC3T3-E1 cells were allowed to adhere in 96-well plates at an initial concentration of 1.0 x 10^4 cells/well for 3 hrs in 100 μl/well ascorbic acid-free α-MEM, after which they were treated with particles at doses of 0.000, 0.075, 0.150, 0.300, and 0.600% v/v for 24, 48, and 72 hrs and then subsequently exposed to BrdU nucleotide for 24 hrs. BrdU uptake by actively proliferating cells was measured using the BrdU Cell Proliferation Assay Kit (Calbiochem). Statistical analysis was performed using t-test with p < 0.05 considered significant.

RESULTS:
Addition of PMMA particles to MC3T3-E1 cells resulted in a dose- and time-dependent increase in LDH release over the 72-hr period (Figure 1). The number of adherent viable cells decreased in a dose-dependent manner, with significant reductions observed at particle doses ≥ 0.150% v/v (Figure 2). Proliferation as assessed by BrdU uptake was unaffected in cells challenged with particles for 24 hrs, but cells challenged with particles for 48 and 72 hrs showed a dose-dependent decrease in BrdU uptake (Figure 3). The TUNEL assay revealed no apoptotic cells at all times and particle doses (Figure 4). Absorbance (450-540 nm) levels of lactate dehydrogenase (LDH), an intracellular enzyme released from dead cells, in culture supernatant at 24, 48, and 72 hrs using the Cytotox96 Non-Radioactive Cytotoxicity Assay (Promega). The number of adherent viable cells was determined each day by hemocytometer cell count with trypan blue staining. A TUNEL assay was performed using the APO-BrdU Apoptosis Detection Kit (eBioscience) for detecting cells with fragmented DNA, a hallmark of apoptosis. To determine particle effects on proliferation, MC3T3-E1 cells were allowed to adhere in 96-well plates at an initial concentration of 1.0 x 10^4 cells/well for 3 hrs in 100 μl/well ascorbic acid-free α-MEM, after which they were treated with particles at doses of 0.000, 0.075, 0.150, 0.300, and 0.600% v/v for 24, 48, and 72 hrs and then subsequently exposed to BrdU nucleotide for 24 hrs. BrdU uptake by actively proliferating cells was measured using the BrdU Cell Proliferation Assay Kit (Calbiochem). Statistical analysis was performed using t-test with p < 0.05 considered significant.

DISCUSSION:
This study has shown that PMMA particles are cytotoxic to MC3T3-E1 osteoprogenitors, as indicated by the dose-dependent increase in LDH release, the dose-dependent decrease in the number of viable cells, and the dose-dependent decrease in BrdU uptake for cells challenged with particles for ≥ 48 hrs. The absence of apoptotic cells as determined by the TUNEL assay indicates that the cells die by necrosis. Clinically, the exposure of osteoprogenitor cells to implant wear debris may lead to a decrease in bone formation by causing cell death in the osteoprogenitor population.

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Figure 1: Dose-dependent release of lactate dehydrogenase (LDH) from MC3T3-E1 cells challenged with PMMA particles over a 72-hr period. N=3, *p < 0.05 vs. control (0.000% PMMA).

Figure 2: Dose-dependent effects of PMMA particles on the number of viable adherent cells over a 72-hr period. N=4, *p < 0.05 vs. control (0.000% PMMA).

Figure 3: Dose-dependent effects of PMMA particles on proliferation as assessed by BrdU uptake. MC3T3-E1 cells were pre-treated with PMMA particles for 24, 48, and 72 hrs (x-axis label) prior to incubation in BrdU for 24 hrs. N=4, *p < 0.05 vs. control (0.000% PMMA).

Figure 4: Left panel: Absence of apoptotic cells in MC3T3-E1 cultures challenged with 0.300% v/v particles for 72 hrs as determined by TUNEL assay. Right panel: positive control with apoptotic cells.