J Mater Sci: Mater Med (2006) 17:667–673
DOI 10.1007/s10856-006-9230-x

Effect of bisphosphonates on the stimulation of macrophages by alumina ceramic particles: A comparison with ultra-high-molecular-weight polyethylene

Alain Petit · Fackson Mwale · John Antoniou · David J. Zukor · Olga L. Huk

Received: 3 January 2004 / Accepted: 21 October 2005

Abstract Wear particle-induced osteolysis and loosening is a critical process that limits the longevity of total hip arthroplasty. Despite their potential value in the management of aseptic loosening, little is known about the cellular response to bisphosphonates (BPs) in the presence of particulate debris. In the present study, we compared the effect of pamidronate and clodronate, two structurally different bisphosphonates, on the induction of TNF-α release by alumina ceramic (Al_2O_3) and ultra-high-molecular-weight-polyethylene (UHMWPE) particles. We also looked, by Trypan blue exclusion, at the viability of J774 mouse macrophages incubated with Al_2O_3 and UHMWPE particles in combination with pamidronate or clodronate. Results showed that pamidronate and clodronate can inhibit UHMWPE particle-induced TNF-α release while they had no effect on Al_2O_3-stimulated TNF-α release. The co-incubation of pamidronate or clodronate and Al_2O_3 had no effect on the induction by Al_2O_3 of poly(ADP-ribose)polymerase (PARP) proteolysis and DNA fragmentation. On the other hand, UHMWPE particles had no effect on these apoptotic markers. However, the co-incubation of pamidronate or clodronate with UHMWPE particles led to the appearance of these markers of apoptosis. Al_2O_3 and UHMWPE particles had no effect on macrophage cell death or the number of macrophages at the end of experiments. Co-incubation of UHMWPE particles with pamidronate and clodronate led to a significant increase in cell death. Interestingly, the number of macrophages co-incubated with particles and pamidronate or clodronate significantly decreased. In conclusion, our results suggest that the effect of BPs on particle-stimulated macrophages is, at least in part, particle composition dependent.

A. Petit · F. Mwale · J. Antoniou · D. J. Zukor · O. L. Huk (✉)
Division of Orthopaedic Surgery, McGill University, Lady Davis Institute for Medical Research, The Sir Mortimer B. Davis—Jewish General Hospital, 3755 Chemin de la Cote Ste-Catherine, Montreal, QC H3 T 1 E2, Canada
e-mail: ohuk@ldi.jgh.mcgill.ca

Introduction

Implant wear producing particulate debris leading to osteolysis and subsequent loosening is a critical process that limits the longevity of total hip arthroplasty. Indeed, wear particles initiate an inflammatory response characterized by macrophage phagocytosis of the particles, leading to the release of mediators that induce bone resorption [1, 2]. Results from our laboratory suggest that bisphosphonates (BPs) can reduce human periprosthetic osteolysis [3]. Others have also shown that alendronate can inhibit wear debris mediated osteolysis in a canine model [4]. BPs are a class of synthetic compounds that are powerful inhibitors of bone resorption both *in vitro* and *in vivo*. They are well tolerated and effective in the treatment of various metabolic bone diseases and skeletal disorders [5, 6]. Despite their potential value in the management of aseptic loosening, little is known about the macrophage response to BPs in the presence of particulate debris. Using a co-culture macrophage/osteoblast model, Horowitz and Gonzales showed that pamidronate was effective in inhibiting resorption stimulated by conditioned medium from macrophages exposed to polyethylene particles [7]. Ibandronate also inhibited UHMWPE-stimulated human macrophage TNF-α release *in vitro* [8]. We demonstrated that the induction of macrophage apoptosis by pamidronate is implicated in the inhibitory effect of this BP on UHMWPE-stimulated TNF-α release [9]. To better understand the interaction between wear particles and BPs, the present study compares the effect of pamidronate

and clodronate, two structurally different BPs, on Al_2O_3 and UHMWPE particle-stimulated macrophage TNF-α release and on macrophage mortality.

Materials and methods

Particles

Alumina ceramic (Al_2O_3; Calcined alumia, Realox reactive grade, mean size of 1.3 μm) particles were obtained from Alcoa (Pittsburgh, PA, USA). They were essentially chosen for their high purity (99.9% Al_2O_3), and their MgO content (fretting additive; <0.02%), which correspond to specifications for hip prosthesis. They were sterilized by ethylene oxide and were left for at least 10 days at room temperature to allow removal of residuals.

Ultra-High-Molecular-Weight Polyethylene particles (UHMWPE, 0.5–2 μm) were obtained from Pfizer (Rutherford, NJ) in 1998. Particles were generated during a hip simulator test using physiological conditions and shipped in suspension in an isopropanol/ deionized water solution. Particles were washed 3 times by centrifugation at 10 000 $\times$ *g* for 5 min in molecular biology grade iscpropanol.

These particles have clinical relevance and their sizes are physiologically pertinent [10–12]. The number of particles used was based on previous work in our laboratory on the differential macrophage response to Al_2O_3 and UHMWPE particles [13].

Level of endotoxins

Particles were suspended in cultured media (without cells) as described in the *Cell Culture* sub-section. The level of endotoxins was determined by the Limulus Amebocyte Lysate Endochrome™ assay (Charles River Endosafe, Charleston, SC) as specified by the manufacturer. Values obtained for media alone were subtracted from the particles results. The level of endotoxins was < 0.005 EU/ml for the incubations with 250 particles/ macrophage (p/m) of Al_2O_3 and < 0.15 EU/ml for the incubations with 25 p/m UHMWPE.

Bisphosphonates

Bisphosphonates (BPs) are compounds derived from pyrophosphate, a normal product of human metabolism. Their biological activity can be modified by altering the structure of the two side chains on the central carbon of their P-C-P group in which the R_2 side chain determines the potency of the molecule [14]. Pamidronate, an amino-BP containing a -NH_2 group in position R_2 (nitrogen-containing BP), and clodronate, a non nitrogen-containing BP with a -Cl group in position R_2, were obtained from Calbiochem (La Jolla, CA). Both BPs were dissolved at 10^{-2}M in culture medium and filtered through 0.22 μm sterile filters before use.

Cell culture

The J774 mouse macrophage cell line (ATCC, Rockville, MD) was used in this *in vitro* model because it has been shown to be capable of phagocytosis as well as cytokine production [15]. Also, this cell line has been described to be morphologically similar to macrophages at the bone-cement interface: both types of cells contain many mitochondria, a relatively small amount of granular endoplasmic reticulum, a well developed Golgi system, and the presence of particles in cytoplasmic vacuoles [15]. Finally, this cell line was shown to respond to Al_2O_3 and UHMWPE particles [13], as well as to BPs [9, 16, 17].

Macrophages were cultured and maintained in RPMI 1640 tissue culture medium supplemented with 5% foetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. For experimental procedures, monolayers of confluent cells were scraped and resuspended in fresh cell culture medium overnight. This allowed the cells to recover from the scraping step that could be damaging to their membrane. After the overnight incubation, the cell concentration was adjusted to 5×10^5 cells/ml and macrophages suspensions were then exposed to particles and BPs. Particles were suspended in culture medium and vortexed for 10 sec at maximal speed just before incubation to avoid clumping. Cells without particles served as negative controls. Incubations were conducted for 24 and 48 h at 37°C in a 5% CO_2 environment. The resulting conditioned supernatants were collected for ELISA tests and frozen at −20°C until TNF-α measurement.

TNF-α release

In the present study, we focused on TNF-α release because it appears to be the most important cytokine produced *in vitro* by J774 macrophages in response to orthopaedic particles [7, 18–20] and it has been suggested that it plays a central role in the induction of implant osteolysis [18, 21]. Concentrations of TNF-α in the supernatants were measured by ELISA (BioSource, Nivelles, Belgium). These enzyme-linked immunosorbent assay kits are murine specific. The detection limits of the assays are 5 to 1250 pg/ml. The percentage of inhibition was calculated by the TNF-α ratio of bisphosphonate-treated cells on untreated cells after subtraction of the control value. Kits are specific enough to avoid cross reactivity of other recombinant cytokines. Statistical significance was calculated using ANOVA followed by Fisher's PLSD comparison test. $P < 0.05$ was considered significant. Each result is the mean $\pm$ SE of 4 experiments performed in duplicate.

PARP expression

Proteins were extracted and analyzed as previously described [9, 13]. Briefly, cells were homogenized in 100 μl of lysis buffer (25 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1% SDS, 10 μl/ml protease inhibitor cocktail (Sigma-Aldrich, Oakville, ON) and incubated for 30 min on ice. Proteins from each sample were denatured at 100°C, loaded on 4–20% acrylamide gels and separated by SDS-PAGE. Proteins were transferred to nitrocellulose membranes and blotting was performed using anti-PARP diluted 1:2000 (Zymed, San Francisco, CA) as primary antibodies with peroxidase-conjugated anti-mouse IgG (Zymed) diluted 1:5000 as secondary antibody. *NEN Renaissance*® luminescence reagents were used for detection. Results are representative of 3 experiments.

DNA fragmentation (DNA laddering)

DNA was isolated as previously described [9, 13] with minor modifications. Briefly, cells were homogenized in 150 μl lysis Buffer (50 mM Tris-HCl, 20 mM EDTA, 1% NP-40, pH 7.4) by pipetting up and down for 10 seconds and centrifuged 5 min at 1600 $\times$ *g* at 4°C. Pellets were re-extracted with lysis buffer and centrifuged. Supernatants were pooled and incubated with RNAse A (Amresco, Solon, OH, USA) and proteinase K (Amresco, Solon, OH, USA) to remove RNA and proteins, respectively. After addition of 1/10 volume of 3 M sodium acetate (pH 5.2), the DNA was precipitated with 2.5 volume ethanol and dissolved in 10 mM Tris-HCl - ImM EDTA, pH 8.0. The samples were loaded onto 1.5% agarose gel containing 50 μg/ml ethidium bromide, and run at 50 volts for about 2 h. DNA was then visualized upon illumination with UV light and photographed using Polaroid 667 film. Results are representative of 3 experiments.

Cell mortality and cell number

After incubation with particles and BPs, cell mortality was obtained by measuring the percentage of dead macrophages by Trypan blue exclusion using a hemacytometer. The number of cells remaining after incubation was also assessed and the cytotoxicity was calculated as a percent of the total number of remaining cells in each test tube over the total number of remaining cells in control. Results are the mean $\pm$ SE of 4 experiments performed in duplicate.

Results

Figure 1 shows the effect of BPs on Al_2O_3 and UHMWPE particle-induced TNF-α release. It has been suggested that endotoxins adsorbed onto particles may play a role in the macrophage response [22, 23]. However, the concentrations of LPS (endotoxin) necessary to stimulate murine macrophage cell lines is more than 50 times higher than the concentrations found in the particles [24, 25]. Therefore, the endotoxin level cannot completely account for the increased TNF-α release by UHMWPE compared to Al_2O_3 particles. Our results also show that pamidronate and clodronate had no effect on TNF-α release stimulated by Al_2O_3 particles/macrophage (p/m), except after 48 h with 125 p/m where a small, but significant, increase was observed (2.45 vs. 2.15 times the control ($p < 0.05$) with or without pamidronate, respectively) (Figure 1A). Pamidronate inhibited UHMWPE-induced TNF-α release by 25% and 21% after 24 h with 10 p/m and 25 p/m, respectively. These inhibitions reached 37% and 19% after 48 h (Figure 1B). Clodronate inhibited UHMWPE-induced TNF-α release by 25% after 24 h with 10 p/m and 25 p/m. These inhibitions reached 46% (10 p/m) and 29% (25 p/m) after 48 h (Figure 1B). In the presence of 25 p/m of UHMWPE, we also observed a dose-dependent effect of the BPs (not shown). Indeed, pamidronate (10 μM) inhibited UHMWPE-induced TNF-α release by 4.0% and 7.4% after 24 h and 48 h, respectively. These inhibitions reached 11.5% and 19.2% with 100 μM after 24 h and 48 h,

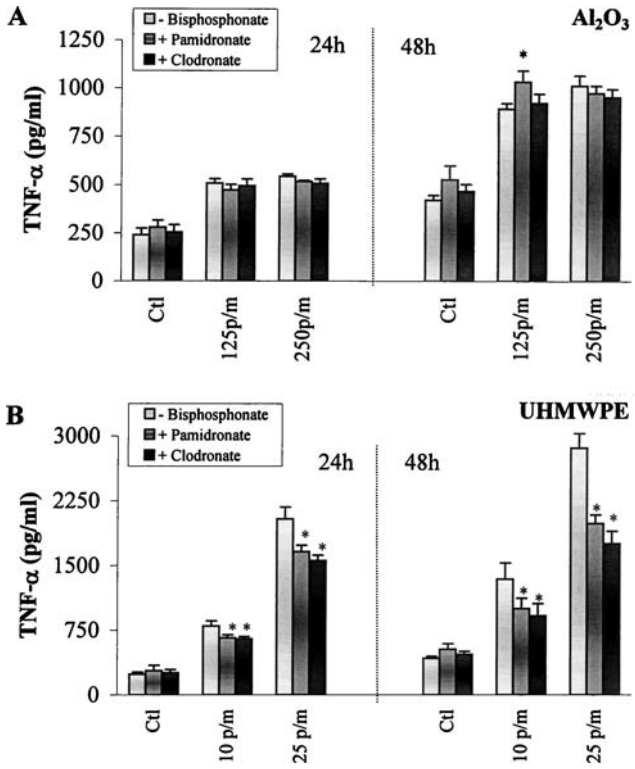

Fig. 1 Effect of pamidronate and clodronate on Al_2O_3 and UHMWPE particle-stimulated TNF-α release. J774 macrophages were co-incubated for 24 and 48 h in the presence of Al_2O_3 (125 and 250 p/m) or UHMWPE (10 and 25 p/m) and 100 μM pamidronate or clodronate. TNF-α release was measured by ELISA. Results are the mean $\pm$ SE of 4 experiments performed in duplicate.
$^*p < 0.05$ vs Al_2O_3 or UHMWPE alone (- bisphosphonate)

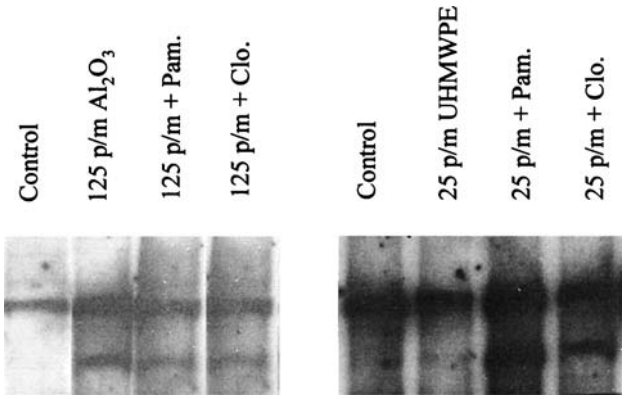

Fig. 2 Effect of pamidronate and clodronate on PARP expression in macrophages stimulated with Al_2O_3 and UHMWPE particles. J774 macrophages were co-incubated for 24 h in the presence of 125 p/m of Al_2O_3 or 25 p/m of UHMWPE and 100 μM pamidronate or clodronate. PARP expression was visualized by Western blot. Results are representatives of 3 experiments.

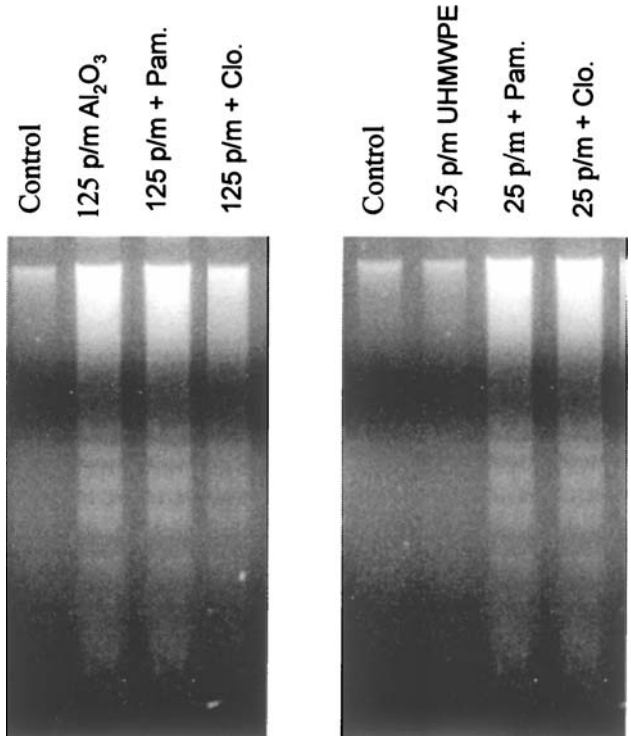

Fig. 3 Effect of pamidronate and clodronate on DNA of macrophages stimulated with Al_2O_3 or UHMWPE particles. J774 macrophages were co-incubated for 48 h in the presence of 125 p/m of Al_2O_3 or 10 p/m of UHMWPE and 100 μM pamidronate or clodronate. DNA was analyzed on 1.5% agarose gel containing ethidium bromide. Results are representatives of 3 experiments.

respectively. Clodronate (10 μM) inhibited UHMWPE-induced TNF-α release by 7.8% and 14.6% after 24 h and 48 h, respectively. These inhibitions reached 25% and 29% with 100 μM after 24 h and 48 h, respectively.

Figure 2 shows the effect of Al_2O_3 or UHMWPE particles co-incubated with pamidronate or clodronate on the expression of PARP, an early event in the induction of apoptosis. Our results show that Al_2O_3 particles alone induced the appearance of the proteolytic PARP fragment (85 kDa) while the co-incubation with pamidronate or clodronate had no additional effect on this proteolysis of PARP by Al_2O_3 particles. UHMWPE particles had no effect on the proteolysis of PARP, the native fragment (113 kDa) remaining intact. Co-incubation of pamidronate or clodronate with UHMWPE particles led to the appearance of the proteolytic PARP fragment after 24 h incubation. Pamidronate or clodronate alone induced the appearance of the proteolytic fragment of PARP (not shown).

Figure 3 shows the effect of Al_2O_3 or UHMWPE particles co-incubated with pamidronate or clodronate on macrophage DNA fragmentation, a late apoptotic event. Our results demonstrate that Al_2O_3 particles alone induced the fragmentation of DNA while the co-incubation with pamidronate or clodronate had no additional effect on this fragmentation of DNA by Al_2O_3 particles. UHMWPE particles had no effect on DNA while the co-incubation of pamidronate or clodronate with UHMWPE particles led to the appearance of a DNA ladder (stimulation of DNA fragmentation) after 48 h of incubation. Pamidronate or clodronate alone induced the fragmentation of macrophage DNA (not shown).

Figure 4 shows the effect of pamidronate and clodronate on macrophage mortality in the presence of 125 p/m of Al_2O_3 and 25 p/m of UHMWPE. Al_2O_3 and UHMWPE particles, as well as pamidronate, had no effect on macrophage mortality. However, 100 μM clodronate significantly increased cell death after 48 h (11.6% vs. 3.7% for control). Co-incubation of UHMWPE particles with 100 μM pamidronate led to a slight but not significant increase in cell death after 48 h while co-incubation of Al_2O_3 particles with the same concentration of pamidronate led to a slight but not significant decrease in cell death. With clodronate (100 μM), there was a significant increase in cell death, reaching 10.5% and 13.2% after 48 h with Al_2O_3 and UHMWPE particles, respectively. Pamidronate and clodronate at 10 μM had no effect on cell mortality (not shown).

Figure 5 shows the effect of pamidronate and clodronate on the number of remaining macrophages in the presence of 125 p/m of Al_2O_3 and 25 p/m of UHMWPE. Co-incubation of particles with 100 μM pamidronate led to a decrease in the total number of macrophages reaching 86% and 82% of control after 24 h and 48 h, respectively. Pamidronate alone had the same effect on the number of macrophages. Co-incubation of UHMWPE particles with 100 μM clodronate led to a decrease in the total number of macrophages reaching 64% and 50% of control after 24 h and 48 h, respectively. The effect of clodronate alone was less important with number of macrophages reaching 77% and 75% of control after 24 h and 48 h, respectively. Al_2O_3 particles induced a slight decrease in macrophage number (84% and 77% of control after

24 h and 48 h, respectively). This decrease was only affected by clodronate and after 48 h (62% of control). Pamidronate and clodronate at 10 μM had no effect on the number of macrophages (not shown).

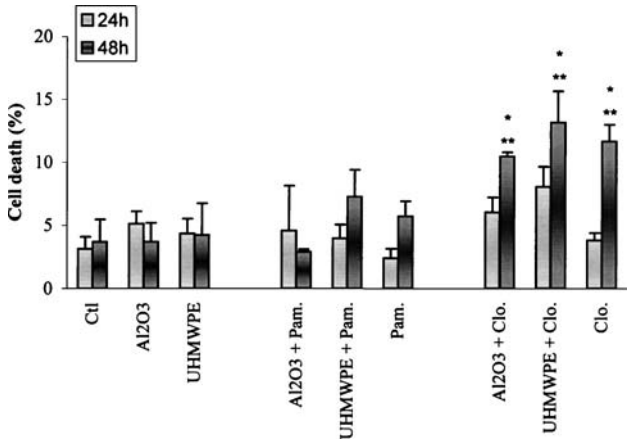

Fig. 4 Effect of pamidronate and clodronate on macrophage cell death in the presence of Al_2O_3 and UHMWPE particles. J774 macrophages were co-incubated for 24 and 48 h in the presence of Al_2O_3 (125 p/m) or UHMWPE (25 p/m) and pamidronate or clodronate (100 μM). Cell death was measured by Trypan blue exclusion using a hemacytometer. Results are the mean ± SE of 4 experiments performed in duplicate.
* $p < 0.05$ vs. control
** $p < 0.05$ vs. 24 h

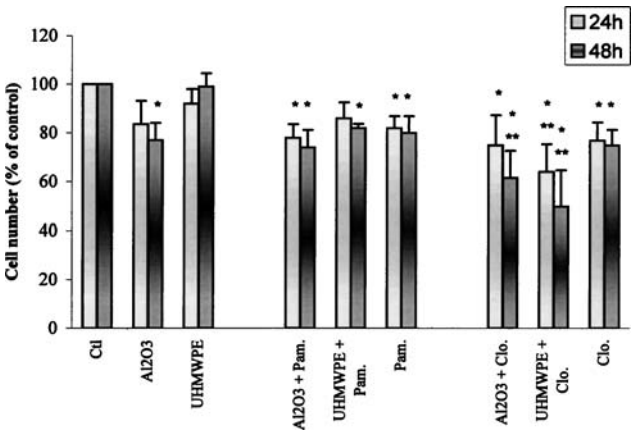

Fig. 5 Effect of pamidronate and clodronate on the number of macrophages in the presence of Al_2O_3 and UHMWPE particles. J774 macrophages were co-incubated for 24 and 48 h in the presence of Al_2O_3 (125 p/m) or UHMWPE (25 p/m) and pamidronate or clodronate (100 μM). The number of cells remaining after incubation was assessed using a hemacytometer. Results are the mean ± SE of 4 experiments performed in duplicate.
* $p < 0.05$ vs. control
** $p < 0.05$ vs. particles alone

Discussion

The evolving role of BPs led to an increase of their use in a variety of clinical causes [26]. With regard to periprosthetic osteolysis, the use of BPs for the treatment and/or prevention of particle-induced osteolysis is far from being a *fait accompli.* Treatment of patients undergoing THA with either alendronate or pamidronate was successful in reducing proximal femoral bone loss often seen in the early postoperative period secondary to stress shielding caused by femoral prosthetic components [27–29]. Unfortunately, the patho-physiology of stress shielding that occurs especially following the implantation of cementless prostheses is totally different and unrelated to wear particle-induced osteolysis. In evaluating potential markers in the assessment and treatment of periprosthetic osteolysis, we demonstrated a higher excretion of urinary telopeptides, a marker of bone resorption, in patients with periprosthetic osteolysis compared to patients with well-fixed prostheses [3]. Furthermore, a six-week treatment course of osteolytic patients with oral alendronate significantly lowered urinary telopeptides excretion, reflecting a decreased bone resorption in response to the drug [3]. This potential beneficial effect of BPs on periprosthetic osteolysis was recently reinforced in a prospective randomized study showing a beneficial effect of alendronate in reducing periprosthetic bone loss at 2 years follow-up [30]. However, the safety and efficacy of BPs in the long term clinical management of osteolysis associated with THA has not been clearly established [31].

The results of the present study show that pamidronate and clodronate, two structurally different BPs, can inhibit UHMWPE particle-induced macrophage TNF-α release while these BPs had no effect on Al_2O_3 particle-induced TNF-α release. Therefore, our results suggest for the first time that the BPs' effect on particle-stimulated TNF-α release is, at least in part, particle composition dependent. We also demonstrated that both pamidronate and clodronate stimulate the cleavage of PARP, an early apoptosis marker, and DNA fragmentation into oligonucleosome length fragments (DNA ladder), a gold standard for the confirmation of apoptosis, suggesting that apoptosis-related events are associated with the inhibition of UHMWPE-induced TNF-α release by these BPs. However, the direct causal link between the induction of apoptosis and the inhibition of TNF-α remain to be investigated. The results also showed that BPs may be cytotoxic and decreased the number of macrophages in the presence of UHMWPE particles.

The results are similar to that observed in murine osteoclasts where pamidronate and clodronate have the same potency in inducing apoptosis [32]. However, pamidronate was reported to be more potent than clodronate (> 20 times) in inducing apoptosis in J774 macrophages [17]. The difference may be due to the presence of particles that may change the ability of macrophages to internalize these BPs. In support of this view, Rader *et al.* [8] demonstrated that etidronate (a first generation non nitrogen-containing BP) and ibandronate (a third generation amino-BP) have similar potency in

inhibiting particle-stimulated TNF-α release. Etidronate, like clodronate, is another short side chain BP with less potency of internalization than the more lipophilic amino-BPs. An alternative explanation is that the solubilization of the BPs in culture media rather than in PBS may also affect the internalization of clodronate and pamidronate by interaction with proteins and lipids in the serum. Nevertheless, our results confirm the potency of BPs to inhibit UHMWPE particle-induced TNF-α release *in vitro*.

In the present study, we used different BP concentrations to demonstrate that the effect of BPs on particle-stimulated macrophages is dose-dependent with significant effects at 100 μM, while their effect was less important at 10 μM. These results parallel studies showing that pamidronate alone induced apparent DNA laddering at 100 μM with low apoptosis at 10 μM [16, 17]. Our results are also in agreement with the effective dose of pamidronate in inhibiting bone growth in a rat growing model [16]. The present results suggest that the induction of apoptosis by BPs or other apoptosis-modulating agents may be an effective way to reduce macrophage response to wear particles. However, our results suggest that the effects of BPs may be particle composition dependent.

The results of our experiments also shed light on the clinical behavior of ceramic THAs. It has been observed that ceramic THAs are associated with less osteolysis than UHMWPE-bearing prosthesis [33]. The reason for this behavior is twofold. Firstly, being a more resistant interface, the ceramic-bearing prosthesis generates less wear particles. Secondly, and as born out by our experiments, ceramic induces less of an inflammatory response than UHMWPE particles [13]. Our results have shown that Al_2O_3 particles alone are capable of inducing macrophage apoptosis, thereby reducing their osteolytic activity.

In the present study, we also used Trypan blue exclusion and the total macrophage number as general markers of cytotoxicity of BPs. Our results suggest that pamidronate and clodronate may be cytotoxic and decreased the number of macrophages in the presence of UHMWPE particles. More specifically, we demonstrated that clodronate seems more toxic than pamidronate in our cell culture model. This is particularly evident for the remaining number of macrophages after incubation in the presence of UHMWPE particles and clodronate. Clodronate is known to deplete macrophages *in vivo* and therefore may be potentially useful in the attenuation of symptoms in diseases related to inflammatory reactions. Indeed, it exerts an excellent preventive effect on joint destruction of chronic antigen-induced arthritis in rats [34, 35]. However, macrophages play a crucial role in the periprosthetic environment and their appearance around any given prosthesis is related to the balance between the rate of production of wear particles, the ability of the tissues to deal with the particles, and the rate of clearance of the particles from the joint [36]. Therefore, their elimination from the periprosthetic environment may be not desirable.

In conclusion, using two structurally different BPs in combination with particles of different composition, our results suggest that the effect of BPs on particle-stimulated macrophages is, at least in part, particle composition dependent. The significance of the decreased macrophage number deserves attention in future studies.

Acknowledgements The authors would like to thank the Orthopaedic Research Fund of the Sir Mortimer B. Davis—Jewish General Hospital for financial assistance.

References

1. D. W. HOWIE, B. MANTHEY, S. HAY and B. VERNON-ROBERTS, *Clin. Orthop. Rel. Res.* **292** (1993) 352.
2. S. GOODMAN, J. S. WANG, D. REGULA and P. ASPENBERG, *Acta Orthop. Scand.* **65** (1994) 157.
3. J. ANTONIOU, O. HUK, D. ZUKOR, D. EYRE and M. ALINI, *J. Orthop. Res.* **18** (2000) 64.
4. A. S. SHANBHAG, C.T. HASSELMAN and H.E. RUBASH, *Clin. Orthop. Rel. Res.* **44** (1997) 33.
5. C. J. ROSEN and C. R. KESSENICH, *Drugs.* **51** (1996) 537.
6. F. R. SINGER, *Current Opin. Orthop.* **7** (1996) 26.
7. S. M. HOROWITZ and J. B. GONZALES, *J. Orthop. Res.* **15** (1997) 50.
8. C. P. RADER, B. BAUMANN, T STERNER, O. ROLF, C. HENDRICH, N. SCHUTZE and E. JACOBS, *Biomediz Technik.* **44** (1999) 135.
9. O. L. HUK, D. J. ZUKOR, J. ANTONIOU and A. PETIT, *J. Orthop. Res.* **21** (2003) 81.
10. P. CAMPBELL, S. MA, B. YEOM, H MCKELLOP, T. P. SCHMALZRIED and H. C. AMSTUTZ, *J. Biomed. Mater. Res.* **29** (1995) 131.
11. J. M. DORLOT, P. CHRISTEL and A MEUNIER, *J. Biomed. Mater. Res.* **23** (A3 Suppl) (1989) 299.
12. S. LEROUGE, O. HUK, L'H. YAHIA and L. SEDEL, *J. Biomed. Mater. Res.* **32** (1996) 627.
13. A. PETIT, I. CATELAS, D. J. ZUKOR and O. L. HUK, *J. Orthop. Res.* **20** (2002) 9.
14. R. G. RUSSELL, M. J. ROGERS, J. C. FRITH, S. P. LUCKMAN, F. P. COXON, HL BENFORD, P. I. CROUCHER, C. SHIPMAN and H. A. FLEISCH, *J. Bone Miner. Res.* **14** (Suppl 2) (1999) 53.
15. S. M. HOROWITZ, S. B. DOTY, J. M. LANE and A. H. BURSTEIN, *J. Bone Joint Surg. [Am].* **75-A** (1993) 802.
16. S. P. LUCKMAN, F. P. COXON, F. H. EBITINO, RGG RUSSELL and M. J. ROGERS, *J. Bone Miner. Res.* **13** (1998) 1668.
17. M. J. ROGERS, K. M. CHILTON, F. P. COXON, J. LAWRY, M. O. SMITH, S. SURI and G. G. RUSSELL, *J. Bone Miner. Res.* **11** (1996) 1482.
18. S. M. ALGAN, M. PURDON and S. M. HOROWITZ, *J. Orthop. Res.* **14** (1996) 30.
19. I. CATELAS, A. PETIT, R. MARCHAND, D. J. ZUKOR, L'H. YAHIA and O. L. HUK, *J. Bone Joint Surg. [Br].* **81-B** (1999) 516.

20. A. PRABHU, C. E. SHELBURNE and D. F. GIBBONS, *J. Biomed. Mater. Res.* **42** (1998) 655.
21. K. D. MERKEL, J. M. ERDMANN, K. P. MCHUGH, Y. ABU-AMER, F. P. ROSS and S. L. TEITELBAUM, *Am. J. Pathol.* **154** (1999) 203.
22. A. U. DANIELS, F. H. BARNES, S. J. CHARKEBOIS and R. A. SMITH, *J. Biomed. Mater. Res.* **49** (2000) 469.
23. R. A. BROOKS, J. A. WIMHURST and N. RUSHTON, *J. Bone Joint Surg. [Br].* **84-B** (2002) 295.
24. T. S. TENGKU-MUHAMMAD, T. R. HUGHES, A. CRYER and D. J. RAMJI, *Cytokine.* **7** (1999) 463.
25. B. GAO and M. F. TSAN, *Biochem. Biophys. Res. Commun.* **317** (2004) 1149.
26. R. L. THERIAULT and G. N. HORTOBAGYI, *Semin. Oncol.* **28** (2001) 284.
27. A. R. LYONS, *Current Opin. Orthop.* **9** (1998) 2.
28. P. K. VENESMAA, H. P. J. KROGER, H. J. A. MIETTINEN, J. S. JURVELIN, O. T. SUOMALAINEN and E. M. ALHAVA, *J. Bone Miner. Res.* **16** (2001) 2126.
29. J. M WILKINSON, I. STOCKLEY, N. F. PEEL, A. J. HAMER, R. A. ELSON, N. A. BARRINGTON and R. EASTELL, *J. Bone Miner. Res.* **16** (2001) 556.
30. A. NEHME, G. MAALOUF, J. L. TRICOIRE, G. GIORDANO, P. CHIRON and J. PUGET, *Rev. Chir. Orthop. Repar. Appar Mot.* **89** (2003) 593.
31. M. D. RIES, *Instruct. Course Lectures.* **52** (2003) 257.
32. D. E. HUGHES, K. R. WRIGHT, H. L. UY, A. SASAKI, Y. YONEDA, G. D. ROODMAN, G. R. MUNDY and B. F. BOYCE, *J. Bone Miner. Res.* **10** (1995) 1478.
33. G. WILLMANN, *Orthopedics.* **21** (1998) 173.
34. P. J. RICHARDS, A. S. WILLIAMS, R. W. GOODFELLOW and B. D. WILLIAMS, *Rheumatol. (Oxford).* **38** (1999) 818.
35. P. OELZNER, A. KUNZE, S. HENZGEN, K. THOSS, G. HEIN, G. STEIN and R. BRAUER, *Inflamm. Res.* **49** (2000) 424.
36. D. W. HOWIE, D. R. HAYNES, S. D. ROGERS, M. A. MCGEE and M. J. PEARCY, *Orthop. Clinics North Am.* **24** (1993) 571.