Bisphosphonate inhibition of bone resorption induced by particulate biomaterial-associated macrophages

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Aseptic loosening of total joint replacements is associated with bone resorption. A heavy infiltrate of foreign body macrophages in response to biomaterial wear particles is commonly found in the fibrous membrane surrounding loose components. It has recently been shown that foreign body macrophages can differentiate into osteoclastic cells. To determine whether pharmacological inhibitors of bone resorption have a role to play in controlling the osteolysis of aseptic loosening, we analyzed the effect of a bisphosphonate, disodium ethane-1, 1-diphosphonate (EHDP) on this process. Murine monocytes and foreign body macrophages (derived from granulomas formed by subcutaneous implantation of particles of prosthetic biomaterials) were co-cultured with UMR106 osteoblast-like cells in the presence of 1,25 dihydroxyvitamin D_3 for 14 days on glass coverslips and bone slices. EHDP significantly inhibited bone resorption in these co-cultures. There was little or no expression of the osteoclast-associated enzyme, tartrate-resistant acid phosphatase (TRAP) in EHDP-treated co-cultures. Addition of EHDP to monocyte-UMR106 co-cultures after the appearance of TRAP-positive cells did not abolish bone resorption, indicating that EHDP, in addition to its known inhibitory effect on osteoclast function, suppresses differentiation of osteoclast precursors. EHDP inhibition of the osteolysis induced by particulate biomaterial-associated macrophages shows that pharmacological inhibition of bone resorption might be used to control the osteolysis of aseptic loosening.

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A heavy macrophage and macrophage polykaryon infiltrate is commonly seen in response to biomaterial wear particles found in periprosthetic tissues surrounding a loose cemented or uncemented prosthesis (Revell 1982, Lennox et al. 1987, Bullough et al. 1988). These wear particle-associated macrophages produce local factors (including prostaglandins and cytokines), which are known to stimulate osteoclastic bone resorption (Murray and Rushton 1990, Amstutz et al. 1992, Glant et al. 1993, Jiranek et al. 1993). Several studies have also suggested that the pathological bone resorption of aseptic loosening around arthroplasty components may be a more direct consequence of the macrophage response to wear particles. It has been shown that human foreign body macrophages derived from the revision hip arthroplasty membrane can directly resorb bone (Athanasou et al. 1992) and that murine inflammatory macrophages responding to polymethylmethacrylate (PMMA) particles can differentiate into osteoclast-like bone-resorbing cells under specific cellular and hormonal conditions of culture (Quinn et al. 1992). As this process can be influenced by both local and systemic inhibitors of bone resorption (Quinn et al. 1994), pharmacological inhibition of macrophage differentiation towards osteoclastic cells could play a role in suppressing the osteolysis around an implant.

Bisphosphonates have been shown to inhibit bone resorption by osteoclasts and macrophages (Chambers 1980, Cecchini et al. 1987, Hughes et al. 1991). The mechanism causing this inhibition is not exactly known, but may involve effects on osteoclast differentiation or function or alteration of the bone matrix. In this study, our general aim has been to ascertain whether inhibition of bone resorption by bisphosphonates could play a role in controlling the osteolysis of aseptic loosening. We have analyzed the effect of a bisphosphonate (EHDP) on the bone resorption seen in an in vitro model of the osteolysis of aseptic loosening, whereby biomaterial particle-associated mac-

rophages are co-cultured with bone stromal cells in the presence of 1,25 dihydroxy vitamin D_3 [1,25 $(OH)_2D_3$]. We have also determined whether bisphosphonates can inhibit mononuclear phagocyte differentiation into osteoclastic bone-resorbing cells and whether this effect is dose-dependent.

Material and methods

Alpha minimal essential medium (Gibco, U.K.) was supplemented with 100 U/mL penicillin, 10 µg/mL streptomycin, 10 mmol L-glutamine (Sigma, U.K.) and 10% heat-inactivated fetal calf serum (Gibco, U.K.), (MEM/FCS). Disodium ethane-1,1-diphosphonate (EHDP) (Procter and Gamble, U.K.) was diluted in MEM/FCS for addition to cell cultures. 1,25-$(OH)_2D_3$ (Solvay Duphar, Netherlands) was dissolved in absolute alcohol and stored at –20 °C. Cloned hormone-responsive osteoblast-like UMR106 cells (subclone: -.01), originally derived from a rat osteosarcoma cell line were generously provided by Professor T.J. Martin (Melbourne, Australia). All incubations were carried out at 37 °C in 5% CO_2.

Preparation of particles

A block of polymerized PMMA (CMW, U.K.), was crushed using a pestle and mortar and the fragments then passed through a series of mesh sieves (Endecotts test sieve, Philip Harris, U.K.) to obtain particle sizes of less than 80 microns. 80-micron particles of ultra-high density polyethylene (HDP) were a kind gift of Corin (U.K.), and titanium particles (1–3 microns) were commercially obtained (Johnson Mathey, Germany). The range of particle size was confirmed by scanning electron microscopy (SEM).

Preparation of granulomas in mice and isolation of biomaterial particle macrophages

Gelatin capsules (Size 2, Farillon Ltd., Essex, U.K.) were loaded with 100 mg of the biomaterial particles then sterilized with ethylene dioxide vapors for 24 hours. Each capsule was implanted subcutaneously into the dorsum of a 6-week-old MF1 mouse. As negative control, mice were implanted with empty gelatin capsules. After 6 weeks, a foreign body granulomatous swelling (approximately 1 cm) formed, at which time each particle-induced granuloma was removed under sterile conditions. Imprints of the cut surface of the granuloma were taken on a clean glass slide for histochemical characterization of inflammatory cells. Part of each lesion was also fixed in formalin and routinely processed for histological examination. The remainder of each lesion was curetted in 2 mL of MEM. The tissue pieces were digested by collagenase (Sigma, U.K.) for 30 minutes; the resultant suspension was centrifuged and the pellet dispersed in MEM to obtain a suspension of cells. This was layered onto bone slices and coverslips, as described below.

Preparation of murine monocytes

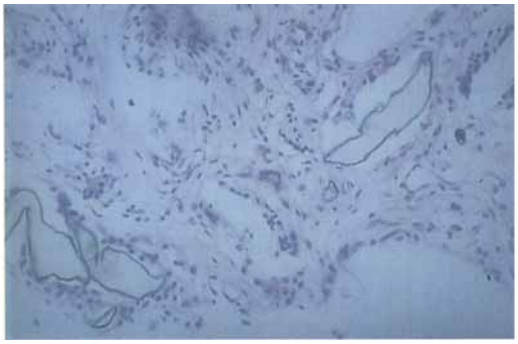
After heart puncture of MF1 mice, blood was collected and diluted 1:4 in HBSS; this was layered over Ficoll-Hypaque (Pharmacia, U.K.), then centrifuged (693 × g) for 20 minutes. The cells at the interface layer were removed, centrifuged (300 × g), and resuspended in HBSS. The number of mononuclear cells in the resulting leucocyte suspension was counted in a hemocytometer. Monocytes were further purified from this cell suspension by allowing the cells to settle and adhere to substrate (cortical bone slices or glass coverslips) for 1 hour at 37 °C; these were then vigorously rinsed to remove non-adherent cells. Adherent cells were characterized for macrophage/osteoclast markers, as described below.

Preparation of UMR106-biomaterial particle macrophage/monocyte co-cultures on bone slices and SEM bone resorption assay

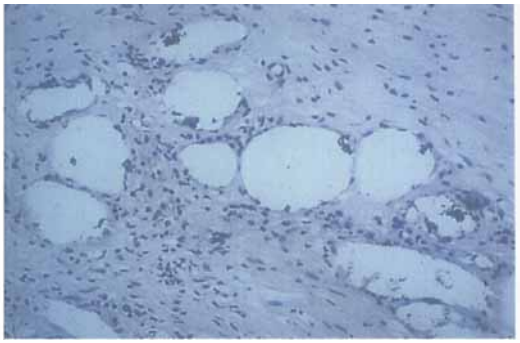
Human cortical bone slices (10 mm^2), prepared as previously described (Athanasou et al. 1989), were placed in 6 mm wells of a 96 well tissue culture plate. UMR106 cells (10^5) were added to each well and cultured on bone slices for 24 h in MEM/FCS at 37 °C. The suspension of biomaterial granuloma-derived macrophages (5000 cells/well) was then settled on the bone slices for 60 minutes. Identical, separate experiments were set up with added monocytes (10^3 cells/well). The bone slices were removed from these wells, washed vigorously in MEM to remove nonadherent cells and placed in 16 mm wells containing 1 mL MEM/FCS with 2×10^{-8}M $1,25(OH)_2D_3$ and 1×10^{-6}M dexamethasone, both of the latter being replenished on every third day of culture. After up to 14 days incubation, the bone slices were removed from culture and processed for SEM, as described below. Identical experiments, as described above, were set up, but with the addition of 100 µg/mL EHDP to the co-cultures.

For each experiment, monocytes and macrophages were also prepared on 6 mm glass coverslips, half of which had been seeded 24 hrs earlier with UMR106 cells. As controls, experiments were set up, using bone slices and coverslips that had not been previously seeded with UMR106 cells. In addition, UMR106 cells were co-cultured alone on bone slices, without the addition of cells isolated from the granulomas. Both cultures on coverslips and all control cultures on

Figure 1. Histology of subcutaneous foreign body granuloma formed by implantation of particles of prosthetic biomaterials, showing the associated heavy macrophage and macrophage polykaryon responses.



Implanted HDP particles (HE, ×250)



Implanted PMMA particles (HE, ×250).

bone slices were maintained with $1,25(OH)_2D_3$ and dexamethasone, both in the presence and the absence of EHDP.

After 1 and 14 days in culture, the bone slices were removed from the wells. To remove the cells coating the surface of the bone, each slice was rinsed in phosphate-buffered saline (PBS) containing 0.2% ethylenediaminetetra-acetic acid (EDTA) before treatment with trypsin solution (0.4%) for 10 mins. Bone slices were rinsed vigorously in distilled water and immersed in 0.25% NH_4OH overnight, then washed in distilled water, dehydrated in graded alcohols and air-dried. The bone slices were mounted onto aluminum stubs, using double-sided sellotape, sputtered with gold and examined, using a Philips SEM 505 scanning electron microscope. A measure of the bone surface area resorbed was made, using Plaqueman assay software on a Macintosh computer.

Time course and dose response to added EHDP on bone resorption in UMR106 - monocyte co-culture

In order to determine the effect of various doses and the time of addition of EHDP on bone resorption, monocytes were added to 10^4 UMR106 cells that were settled 24 hrs earlier on bone slices. After washing, these bone slices were placed in 16mm wells containing MEM-FCS with added 2×10^{-8}M $1,25-(OH)_2D_3$ and cultured for 14 days. 300 µg/mL of EHDP was added to these co-cultures (at the commencement and after 1, 4 and 7 days of culture) before being fixed and prepared for examination by SEM (6 bone slices per time-point of incubation; five experiments). EHDP was also added at various concentrations (3 µg/mL, 30 µg/mL, 100 µg/mL and 300 µg/mL) at the commencement of the cultures (day 0) to determine the dose-response.

Cell cultures on coverslips: characterization of cells isolated from the granulomas

Coverslips containing UMR106-monocyte/macrophage co-cultures were removed after 1 and 7 days of incubation. They were fixed in 10% buffered formalin and histochemically stained for the osteoclast-associated enzyme tartrate-resistant acid phosphatase (TRAP) (Minkin, 1982), using a kit from Sigma (U.K.). In addition, coverslips were immunohistochemically stained by the indirect immuno-peroxidase method with the anti-macrophage antibody F4/80 (Austyn and Gordon, 1981); the F4/80 antigen is known to be expressed by monocytes and macrophages, but is not present on osteoclasts. The imprints from the cut surface of the biomaterial particle-induced granulomas were stained by a similar procedure.

The Mann-Whitney U-test was used to determine the statistical significance of results.

Results

Histology of the subcutaneous granulomas formed in response to biomaterial particles

Subcutaneous lesions formed in response to the various implanted biomaterials showed a foreign body granulomatous response, biomaterial particles lying within or surrounded by macrophages and macrophage polykaryons (Figure 1). Imprint preparations made on glass slides of the cut surface of the granulomas showed numerous mononuclear and multinucleated cells; these were positive for the macrophage marker F4/80, but negative for the osteoclast-associated enzyme TRAP. No granulomatous response was seen when empty gelatin capsules were implanted subcutaneously.

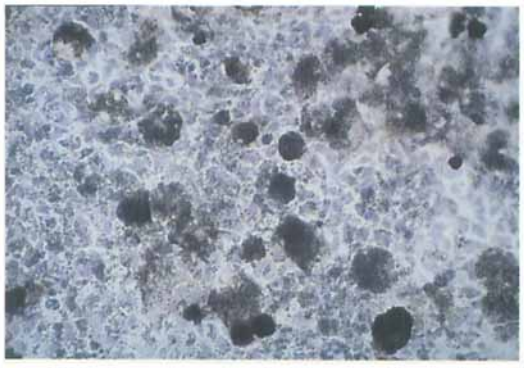


Figure 2. Indirect immunoperoxidase staining for F4/80 (macrophage-associated) antigen, showing positive (brown-staining) reaction of PMMA granuloma-derived mononuclear phagocytes in co-culture with UMR106 stromal cells which are unstained (×400).

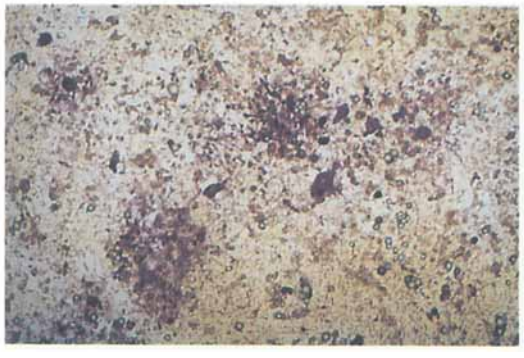


Figure 3. a) Histochemical staining for TRAP of 7 day co-culture of UMR106-PMMA granuloma-derived macrophages showing several TRAP-positive clusters formed in co-culture as well as numerous scattered TRAP-positive cells (×100).

b) Negative control, 7-day co-culture incubated in the absence of $1,25(OH)_2D_3$ and dexamethasone (× 100).

Characterization of cells isolated from biomaterial particle-induced granulomas

After being settled onto glass coverslips, cells isolated from the granulomas formed in response to the various implanted biomaterial particles were identified as macrophages because they were positive for F4/80 (Figure 2), but entirely negative for TRAP upon isolation and after 1 day in culture; this pattern of staining was seen both in the presence and the absence of UMR106 cells. After 7 days in co-culture with UMR106 cells in the presence of $1,25\text{-}(OH)_2D_3$, but without bisphosphonates, coverslips contained scattered F4/80 positive cells, as well as numerous scattered TRAP-positive cells, many of which were located in clusters (Figure 3). Numerous F4/80 positive cells but few, if any, TRAP-positive cells were found in co-cultures to which EHDP had been added. TRAP-positive cells were not seen in control cultures of macrophages to which stromal cells or $1,25\text{-}(OH)_2D_3$ had not been added.

Bone resorption by biomaterial particle-induced macrophages in co-cultures

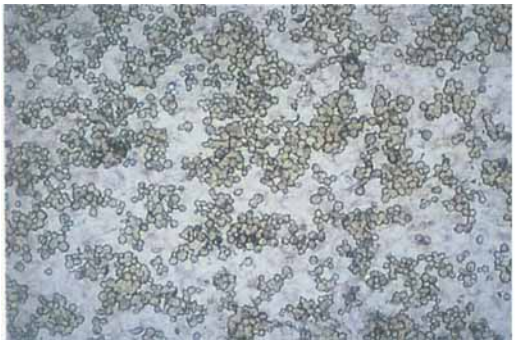
The SEM bone resorption assay showed no evidence of bone resorption on bone slices upon which macrophages and UMR106 cells had been co–cultured for 24 hours. However, numerous lacunar resorption pits were found on bone slices to which macrophages isolated from lesions containing implanted PMMA, HDP and titanium had been cultured for 7 and 14 days in the presence of UMR106 cells and $1,25\text{-}(OH)_2D_3$ (Figure 4). These appeared as single resorption pits or confluent areas of lacunar resorption on the bone surface.

No resorption pits were found in bone slices after 14 days of incubation when EHDP (100 µg/mL) was added at the commencement of co-cultures containing PMMA, HDP or titanium granuloma-derived macrophages. Culture of either macrophages or UMR106 cells alone on bone slices did not result in bone resorption.

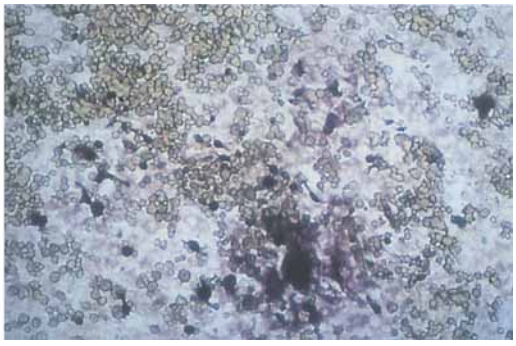
Time course and dose response of EHDP inhibition of bone resorption in monocyte-UMR106 co–cultures

In order to determine whether bisphosphonates had an effect on mononuclear phagocyte differentiation towards bone-resorbing cells, we added EHDP to

Figure 4. Histochemical staining for TRAP of 7-day co-culture of monocytes and UMR106 cells.



EHDP-treated co-culture showing absence of TRAP clusters (×250).



Positive control showing numerous TRAP-positive cells including a single cluster (×250).

monocyte-UMR106 co-cultures on glass coverslips and bone slices which were set up identically with those containing biomaterial particle-induced macrophages. Addition of 100 μg/mL of EHDP both at the commencement (day 0) and 24 hours after setting up monocyte-UMR106 co-cultures resulted in complete inhibition of bone resorption (Figure 5); this was associated with the absence of TRAP staining in co-cultures on glass coverslips. When the same concentration of EHDP was added at day 4, some single resorption pits were seen on bone slices, but the area of bone resorbed was considerably less than in control co-cultures to which EHDP had not been added. These cultures contained a few TRAP-positive cells, but many fewer than in control-untreated co-cultures. However, when EHDP was added on day 7 of co-cultures, minimal inhibition of bone resorption was seen and staining for TRAP was similar to that seen in control co-cultures. Bone resorption was completely abolished by the addition of EHDP to these cultures at concentrations of 100 μg/mL and above (Figure 6). Resorption pit formation was also significantly inhibited but not abolished when EHDP was added at 30 μg/mL but no significant inhibitory effect was noted when EHDP was added at concentration of 3 μg/mL.

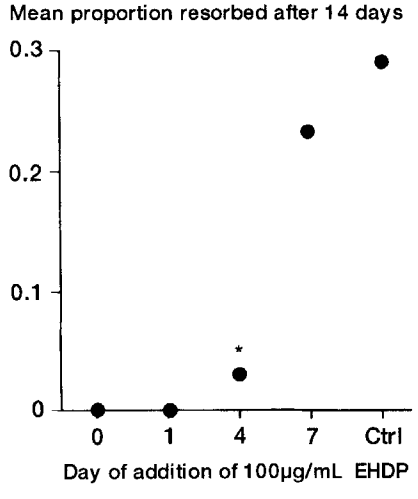


Figure 5. Percentage of the bone slice surface area resorbed, measured after 14 days' incubation, following addition of 100μg/ml EHDP at the commencement (day 0) and 1, 4, and 7 days after monocyte-UMR106 co-cultures were set up on bone slices. * $p < 0.02$ relative to control (untreated) co-cultures.

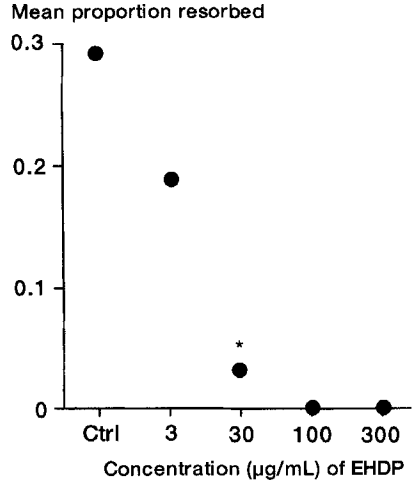


Figure 6. Percentage of the bone slice surface area resorbed 14 days after addition of varying concentrations of EHDP at commencement of monocyte-UMR106 co-cultures. * $p < 0.02$ relative to control (untreated) co-cultures.

Discussion

The subcutaneous granulomas formed in response to implanted biomaterial particles contained a heavy macrophage and macrophage polykaryon infiltrate similar to that seen in the fibrous tissue membrane surrounding loose arthroplasty components (Revell 1982, Lennox et al. 1987, Bullough et al. 1988). Numerous clinical studies have noted the association between the osteolysis of aseptic loosening and the presence of biomaterial wear particles (for review, see Harris 1994, Amstutz et al. 1992). The severity and rapidity of onset of aseptic loosening are closely associated with the degree of the foreign body macrophage response induced by these wear particles (Harris et al. 1976, Santavirta et al. 1990a, b). In our study, we found that one way in which foreign body (particulate biomaterial-associated) macrophages can directly contribute to the osteolysis of aseptic loosening is by differentiation into osteoclast-like cells, which are capable of extensive lacunar bone resorption. We also found that this differentiation and consequent bone resorption can be inhibited and even abolished by the addition of the bisphosphonate, EHDP.

Unlike osteoclasts, particulate biomaterial-associated macrophages isolated from subcutaneous granulomas were F4/80-positive and TRAP-negative; they did not form resorption pits when incubated either alone (for up to 14 days) or in the presence of UMR106 cells, after 1 day in culture (Chambers et al. 1984). However, after co-culture for 7 days with UMR106 osteoblastic cells in the presence of 1,25 $(OH)_2D_3$, these macrophages differentiated into TRAP-positive osteoclast-like cells capable of extensive lacunar bone resorption. As confirmed in our study, murine monocytes show a similar pattern of cytochemical and functional differentiation when co-cultured with UMR106 cells (Udagawa et al. 1990, Quinn et al. 1994). It is known that in hemopoietic spleen and marrow cell cultures in which osteoclastic cells develop, osteoclast progenitors undergo proliferation in the first 4 days of culture and differentiate thereafter into osteoclasts, at which time bone resorption is evident (Takahashi et al. 1988, Tanaka et al. 1993). A similar process is seen when monocytes are co-cultured with UMR106 cells, bone resorption not being seen until the 4th day of co-culture and increasing markedly thereafter, at least until day 7 of co-culture (Quinn et al. 1994).

In our study, we found that the number of TRAP-positive cells formed in monocyte-UMR106 co-cultures depended largely on the time at which EHDP was added. When added at the commencement of co-cultures, TRAP activity was absent and bone resorption was abolished but, when added after 4 days, both TRAP activity and bone resorption were significantly inhibited compared to untreated control co-cultures; in monocyte-stromal cell co-cultures maintained for 7 days before the addition of EHDP, numerous TRAP-positive cells were found on coverslips and extensive bone resorption was seen on bone slices. This chronological pattern of EHDP suppression of the expression of osteoclastic cytochemical and functional markers suggests that EHDP acts to inhibit the phase of proliferation and/or osteoclastic differentiation of precursor cells found in this mononuclear phagocyte population. When added after osteoclastic cells are known to have formed in these cultures, bone resorption is not significantly inhibited.

The mechanism whereby bisphosphonates inhibit bone resorption is not known. Bisphosphonates induce a dose-dependent suppression of bone resorption, as judged by kinetic and indirect chemical measurements (Schlesinger and Blair 1992). This is paralleled by our own finding, showing a dose-dependent inhibitory effect of EHDP inhibition in monocyte-UMR106 co-cultures. Administration of bisphosphonates leads to inhibition of migration of osteoclast precursors to the bone surface (Lowik et al. 1988); it also inhibits their fusion to form multinucleated cells and expression of TRAP activity (Boonekamp et al. 1986, Lowik et al. 1988). Bisphosphonates also preferentially inhibit the proliferation of macrophages in concentrations that do not affect the viability of non-proliferative cells (Cecchini and Fleisch 1990). As inflammatory granulomas are known to contain mononuclear phagocytes which are capable of further proliferation (Spector and Lykke 1966), it is possible that the inhibitory effect of EHDP is primarily directed against such relatively immature mononuclear phagocytes present within the subcutaneous biomaterial particle granulomas; upon isolation, these cells appear to be capable of further proliferation and differentiation towards cells of the osteoclast lineage; the culture conditions we employ provide all the elements required for formation of bone-resorbing cells viz. bone-derived stromal cells, $1,25(OH)_2D_3$, bone itself and a suitable precursor population—i.e., mononuclear phagocytes.

The possibility that bisphosphonates also act through other mechanisms to inhibit bone resorption by other mechanisms via biomaterial particle-associated macrophages is not excluded by our findings. It is well recognized that pretreating devitalized bone particles with bisphosphonates suppresses macrophage-mediated and osteoclastic bone resorption (Chambers 1980, Reitsma et al. 1982, Carano et al. 1990, Flanagan and Chambers 1991). Boonekamp et

al. (1986) have suggested that bisphosphonates inhibit bone resorption by two different mechanisms, both of which require binding of resorbing cells to the mineralized bone matrix, a higher concentration of bisphosphonates being required to inhibit bone resorption by preformed osteoclasts than to inhibit osteoclast formation from mononuclear precursor cells. Another possible mode of action whereby bisphosphonates could inhibit bone resorption is through their ability to modulate bone cell production of cytokines such as interleukin 1 and tumor necrosis factor (Hughes et al. 1991); both of these factors are present at the bone-implant interface and have been implicated in the pathogenesis of bone resorption in aseptic loosening (Amstutz et al. 1992, Haynes et al. 1993, Jiranek et al. 1993).

Pharmacological inhibitors of bone resorption, such as bisphosphonates, may have a role to play in preventing or controlling the osteolysis of aseptic loosening, inhibition of differentiation of osteoclast precursors present in the biomaterial wear-particle-associated macrophage population providing the basis of a therapeutic strategy which would inhibit the osteolysis around a loose prosthesis and increase its longevity. Bisphosphonates are known to be capable of influencing bone resorption without substantially altering non-bone calcium and phosphate regulation; this gives them unique therapeutic potential in controlling various osteolytic bone diseases (Schlesinger and Blair 1992). Bisphosphonates, or other agents directed at inhibiting bone resorption, might conceivably be incorporated into bone cements (as antibiotics are currently), directly coated onto uncemented prosthetic components or administered orally to prevent or to inhibit the differentiation and function of bone resorbing cells in aseptic loosening.

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References

Amstutz H C, Campbell P, Kossovsky N, Clarke I C. Mechanism and clinical significance of wear debris-induced osteolysis. Clin Orthop 1992; 276: 7-18.

Athanasou N A, Wells C A, Quinn J, Ferguson D J P, Heryet A, McGee J O'D. The origin and nature of stromal osteoclast-like multinucleated giant cells in breast carcinoma: implications for tumour osteolysis and macrophage biology. Br J Cancer 1989; 59: 491-8.

Athanasou N A, Quinn J, Bulstrode C J K. Resorption of bone by inflammatory cells derived from the joint capsule of hip arthroplasties. J Bone Joint Surg (Br) 1992; 74: 57-62.

Austyn J M, Gordon S. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur J Immunol 1981; 11: 805-15.

Boonekamp P M, van der Wee-Pals J A, van Wijk-van Leenep M M L, Thesing C W, Bijovet O L M. Two modes of action of bisphosphonates on osteoclastic resorption of mineralised matrix. Bone Mineral 1986; 1: 27-39.

Bullough P G, DiCarlo E F, Hansraj K K, Neves M C. Pathologic studies of total joint replacement. Orthop Clin North Am 1988; 19: 611-25.

Carano A, Teitelbaum S L, Konsek J D, Schlesinger P H, Blair H C. Bisphosphonates directly inhibit the bone resorption activity of isolated avian osteoclasts in vitro. J Clin Invest 1990; 85: 456-61.

Cecchini M G, Fleisch H. Bisphosphonates in vitro specifically inhibit, among the hematopoietic series, the development of the mouse mononuclear phagocyte lineage. J Bone Miner Res 1990; 5: 1019-27.

Cecchini M G, Felix R, Fleisch H, Cooper P H. Effect of bisphosphonates on proliferation and viability of mouse bone marrow- derived macrophages. Bone Miner Res 1987: 2: 135-42.

Chambers T J. Diphosphonates inhibit bone resorption by macrophages in vitro. J Pathol 1980; 132: 255-62.

Chambers T J, Revell P A, Fuller K, Athanasou N A. Resorption of bone by isolated rabbit osteoclasts. J Cell Science 1984; 66: 383-99.

Flanagan A M, Chambers T J. Inhibition of bone resorption by bisphosphonates: interactions between bisphosphonates, osteoclasts and bone. Calcif Tissue Int 1991; 49: 407-15.

Glant T T, Jacobs J J, Molnar G, Shanbhag A S, Valyon M, Galante J O. Bone resorption activity of particulate-stimulated macrophages. J Bone Miner Res 1993; 8: 1071-9.

Harris W H. Osteolysis and particle disease in hip replacement: a review. Acta Orthop Scand 1994; 65: 113-23.

Harris W H, Schiller A L, Scholler J-M, Freiberg R A, Scott R. Extensive localized bone resorption in the femur following total hip replacement. J Bone Joint Surg (Am) 1976; 58: 612-7.

Haynes D R, Rogers S O, Hay S, Pearce M J, Howie D. The differences in toxicity and release of bone resorbing mediators induced by titanium and cobalt-chromium alloy wear particles. J Bone Joint Surg (Am) 1993; 75: 825-33.

Hughes D E, Mian M, Guilland-Cumming D F, Russell R G G. The cellular mechanism of action of bisphosphonates. Drugs Exp Clin Res 1991; 17: 109-14.

Jiranek W A, Machado M, Jasty M, Jevsevar D, Wolfe H J, Goldring S R, Goldberg M J, Harris W H. Production of cytokines around loosened cemented acetabular components. J Bone Joint Surg (Am) 1993; 75: 863-79.

Lennox D W, Schofield B W, McDonald D F, Riley L H. A histological comparison of aseptic loosening of cemented, press-fit, and biologic ingrowth prostheses. Clin Orthop 1987; 225: 171-91.

Lowik C W G M, van der Pluijm G, van der Wee-Pals L J A, Bloys van Treslong-de Groot H, Bijvoet O L M. Migration and phenotypic transformation of osteoclast precursors into mature osteoclasts: the effect of a bisphosphonate. J Bone Miner Res 1988; 3: 185-92.

Minkin C. Bone acid phosphatase: Tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif Tissue Int 1982; 34: 285-90.

Murray D W, Rushton N. Macrophages stimulate bone resorption when they phagocytose particles. J Bone Joint Surg (Br) 1990; 72: 988-92.

Quinn J M, Joyner C, Triffitt J T, Athanasou N A. Polymethylmethacrylate-induced inflammatory macrophages resorb bone. J Bone Joint Surg (Br) 1992; 74: 652-8.

Quinn J M W, McGee J O'D, Athanasou N A. Cellular and hormonal factors influencing monocyte differentiation into osteoclastic bone-resorbing cells. Endocrinology 1994; 134: 2416-23.

Reitsma P H, Teitelbaum S L, Bijvoet O L M, Kahn A J. Differentiation of bisphosphonates (3-amino-1-hydroxypropylidene)-1, 1 bisphosphonate (APD) and disodium dichloromethylidene bisphosphonate (Cl_2 MDP) on rat macrophage-mediated bone resorption in vitro. J Clin Invest 1982; 70: 927-33.

Revell P A. Tissue reactions to joint prostheses and the products of wear and corrosion. In: Current trends in pathology (Ed. Berry C). Springer-Verlag Berlin 1982; 73-101.

Santavirta S, Hoikka V, Eskola A, Kontinnen Y, Paavilainen T, Tallroth V. Aggressive granulomatous lesions in cementless total hip arthroplasty. J Bone Joint Surg (Br) 1990a; 72: 980-4.

Santavirta S, Konttinen T Y, Bergroth V, Eskola A, Tallroth K, Lindholm T S. Aggressive granulomatous lesions associated with hip arthroplasty: immunopathological studies. J Bone Joint Surg (Am) 1990b; 72: 252-8.

Schlesinger P H, Blair H C. Bisphosphonates. In: Biology and physiology of the osteoclast. (Eds. Rifkin B R, Gay C V) CRC Press, Boca Raton 1992; 397-419.

Spector W, Lykke A W J. The cellular evolution of inflammatory granulomata. J Pathol 1966; 92: 163-72.

Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseby J M, Martin T J, Suda T. Osteoblastic cells are involved in osteoclast formation. Endocrinology 1988; 123: 2600-2.

Tanaka S, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stanley E R, Kurokawa T, Suda T. Macrophage colony stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. J Clin Invest 1993; 91: 257-63.

Udagawa N, Takahashi N, Akatsu T, Tanaka H, Sasaki T, Nishihara T, Koga T, Martin T J, Suda T. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. Proc Natl Acad Sci USA 1990; 87: 7260-4.