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BENIGN CELLULAR RESPONSES IN RATS TO DIFFERENT WEAR PARTICLES IN INTRA-ARTICULAR AND INTRAMEDULLARY ENVIRONMENTS

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We examined the cellular responses to various particles injected into the knees and the intramedullary femoral cavities of rats in the presence of polymethylmethacrylate (PMMA) plugs.

The intra-articular particles were mainly ingested by synovial fibroblasts. Increased numbers of macrophages were not detected and there was only a slight increase in synovial thickness.

Cellular responses in the intramedullary space were similarly mild and bone resorption around the PMMA plug did not occur. Bone formation was inhibited only by polyethylene particles.

In contrast to current views, our study shows that wear particles *per se* do not initiate bone resorption.

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Prosthetic loosening caused by bone resorption is the most significant problem in both cemented and cementless total hip replacements. Many studies have shown cellular reactions and have led to the suggestion of a chronic inflammatory response to wear particles.¹⁻⁴ Histochemical studies have demonstrated high levels of metalloproteinases,^{5,6} prostaglandin E₂,^{7,8} elastase⁹ and cathepsins B and G^{10,11} in the interface tissue and in vitro studies have shown the ability of particle-activated macrophages to resorb bone by

the production of proteolytic enzymes.¹²⁻¹⁷ Fibroblasts have been reported to show proliferative responses in vitro after exposure to metallic particles¹⁸ and other studies have suggested a role for several cytokines such as tumour necrosis factor α , interleukin-1 and interleukin-6, which are strong inducers of bone resorption.¹⁹

The in vitro studies seem to indicate an important role for wear particles in the loosening process. In animal models, however, the cellular responses to the implantation of particulate materials in soft tissues,²⁰⁻²³ joint spaces²⁴⁻²⁹ and intramedullary cavities³⁰⁻³⁴ have been shown to be very heterogeneous. The site of implantation and the type and the size of the particles used are important.

It has been generally assumed that the formation of particulate debris from prostheses is an important trigger of bone resorption,^{35,36} but the considerable variation in the methods and models used in these studies makes it difficult to determine the cellular responses of host tissues in vivo to the presence of different types of particle.

Our aim therefore was to compare the cellular responses in vivo to the various types of wear particle which are found around loose total hip prostheses.

MATERIALS AND METHODS

Preparation of the particles. We used several different types of particle. Ultra-high-molecular-weight-polyethylene (UHMWPE) particles from hip simulators were supplied by the Joint Replacement Institute, Los Angeles, California. They are described as being similar in size (mean $1.5 \times 0.25 \mu\text{m}$) and appearance to those found in periprosthetic tissues.^{1,37} Single molecules of high-density polyethylene (HDPE) were synthesised and characterised by SEM to show that their size was uniform ($2 \times 0.2 \mu\text{m}$). Particles of cobalt chromium (Co-Cr) and titanium-alloy (Ti) were prepared by a milling method using material from retrieved prostheses. Sorting of the particles by size was done by differential centrifugation. Particle size was determined by SEM and those of up to $2 \mu\text{m}$ in size were selected. Zirconium oxide (Zr) particles used to render bone cement radio-opaque were obtained by dissolving PMMA in acetone and subsequently grinding and sieving the residue to

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obtain particles in the desired size range (up to 2 μm). Finally, latex particles (polystyrene beads in a 10% aqueous suspension) with a uniform size of 0.5 μm were purchased from Sigma-Aldrich, Bornem, Belgium.

All the particles were suspended in a 0.9% saline solution, with the exception of those of HDPE and UHMWPE which were suspended in a mixture of isopropanol and distilled water (30:70 v/v) to prevent them from adhering to each other. The particle concentrations of all suspensions were similar (10^9 to 10^{10} particles per ml). Finally, the suspensions were sterilised by gamma irradiation (mean 22 kGy, cesium sources).

Experimental procedures. We used 99 young adult male Wistar rats with a mean weight of 275 g. The protocol had been approved by the Animal Research Committee of the University of Amsterdam.

In the first part of our study we used 48 rats divided into eight groups of six. Six groups received two intra-articular injections of one of the six particle suspensions into the right knee with a two-week interval between injections. The remaining two groups, which acted as controls, had injections of 0.9% saline or 30% isopropanol in distilled water. The volume injected each time was 50 to 75 μl containing 5 to 10×10^7 particles. Three rats did not receive any intra-articular injection. All the animals were killed two weeks after the last injection with an overdose of barbiturates.

In the second part of the study, we performed a lateral arthrotomy of the right knee of the remaining 48 rats under general anaesthesia. A hole of 1.1 mm diameter was made in the intercondylar notch with a water-cooled low-velocity drill. The intramedullary cavity was hand-reamed and rinsed with sterile iced 0.9% saline solution until the bleeding had stopped. In each group of six animals the cavity was filled with one of the particle suspensions or control solutions. Immediately afterwards, a preformed sterile PMMA cylinder (1.0 mm diameter and 1 cm length) was placed in the cavity which was sealed with bonewax. Four weeks later the animals were killed with an overdose of barbiturates.

Histochemical procedures. Undecalcified cryostat sections (8 μm) were prepared.^{38,39} Morphological studies were performed after staining briefly with solutions of Toluidine Blue or Giemsa. Photomicrographs were made immediately using a Vanox T light microscope (Olympus, Tokyo, Japan). Immunohistochemical detection of cells of the macrophage lineage was performed using the monoclonal antibodies ED1, ED2 and ED7,⁴⁰⁻⁴² which recognise specifically different populations of the rat monocyte lineage: ED1 recognises monocytes, macrophages and dendritic cells, ED2 tissue macrophages and ED7 macrophages, granulocytes and natural killer cells.⁴³ Detection of osteoclasts in the intramedullary spaces was performed after staining for tartrate-resistant acid phosphatase (TRAP).³⁸

Electron microscopy. After chemical fixation and decalcification, joints and femora were treated with osmium

tetroxide and embedded in LX 112 epoxy resin according to routine procedures. Semi-thin sections (1 μm) were stained with an aqueous solution of 0.1% Toluidine Blue. Ultrathin sections of selected areas were stained with uranyl acetate and with lead citrate before examination with a EM 10C electron microscope (Zeiss, Oberkochen, Germany).

Morphometric analysis. The thickness of the synovium, the number of TRAP-positive osteoclasts and the formation of new bone around the PMMA plugs were determined using a Quantimet Q 500 microscope (Leica, Cambridge, UK) with a 2.5 objective. Two Giemsa-stained sections taken at least 50 μm apart were selected from each animal for analysis. The thickness of the synovium, perpendicular to the surface, was measured at five randomly-selected sites in each section. The osteoclasts were counted in an area between both cortical boundaries and the proximal 1.5 mm of the PMMA implant. The total area analysed was approximately 3 mm^2 for each section. Formation of new bone was measured in sections taken parallel to the axis of the PMMA implants and expressed as the mean percentage of the surface of the implants covered by bone. Statistical analysis of the quantitative data was performed with one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple-comparisons test.

RESULTS

Effects of the intra-articular administration of particles.

The knees of the control animals which had been injected with 0.9% saline solution or 30% isopropanol in water, respectively, had normally-structured synovial tissue (Fig. 1a). The superficial synovial layer was unicellular and consisted of macrophages and fibroblasts, whereas the deeper layers had mainly fibroblasts with few macrophages and mast cells. There was no evidence of inflammation. The animals that had received particle suspensions had synovial tissue similar to that of the control groups without any inflammatory response (Figs 1b and 1c). The thickness of the synovial layer was increased, however, especially in response to HDPE and UHMWPE particles, but the increase was small. Compared with arthritic rat knees the thickness was only slightly increased (Fig. 2). Fibroblasts in the synovium had phagocytosed considerable amounts of all of the particles as was confirmed by electron microscopy (Fig. 3). There were no signs of cell lysis or death. Macrophages were also observed to have ingested particles, but there was no increase in the numbers of these cells (Fig. 4). The fibroblasts remained the main cell type in the synovium, and foreign-body giant cells or increased amounts of mast cells were not observed.

Effects of intramedullary administration of particles.

The control groups showed that a shell of new bone had formed around the PMMA implants after four weeks covering approximately 50% of the total surface (Fig. 5a; Table I). There was direct contact between the bone and PMMA without intervening fibrous tissue. Similar amounts of new

Fig. 1

Photomicrographs showing the appearance of the synovium (s) in rat knees after injection of 0.9% saline (a), Co-Cr (b) and UHMWPE (c). The thickness of the synovium (s) was increased in the presence of UHMWPE. Co-Cr particles are indicated by arrows; ca, cartilage (Giemsa; bar = 100 μ m).

Fig. 2

Graph showing the mean thickness of the synovial layer and the effects of different types of particle compared with knees during the acute phase of inflammation^{39,40} and control knees without injections or injected with 0.9% saline or 30% isopropanol. The T-bars represent the standard deviation (*, significant difference between test and non-injected control; +, significant difference between test and saline and isopropanol control).

bone around the PMMA implant were also seen in the groups which had received Co-Cr, Ti, Zr or latex particles in the intramedullary canal (Fig. 5b; Table I). When Co-Cr particles had been used, some areas of fibrous tissue were observed at the PMMA-bone border but morphometric analysis showed no significant difference when compared with the control groups (Table I). Dispersed particles were present around the implants and between bone trabeculae. Most of the particles were found extracellularly but near the PMMA implant they had been ingested by fibroblast-like cells. Macrophages or foreign-body giant cells were rarely seen. There was no increase in the number of osteoclasts compared with the control groups (Table I). Notable differences were seen in the groups which had received UHMWPE and HDPE particles. Very little new bone had formed around the PMMA implant, the cylinder being surrounded by fibrous tissue without evidence of inflammation, macrophage activity or giant-cell infiltration (Fig. 5c; Table I). Particles were found either extracellularly or intracellularly in fibroblast-like cells around the PMMA implant. There was no increase in the number of osteoclasts or evidence of activity (Howship lacunae) similar to the other four particle groups.

DISCUSSION

To our knowledge there have been no animal studies in which bone resorption could be attributed to the presence

Fig. 3

Transmission electron micrograph of a section of synovium showing UHMWPE particles (arrowheads) intracellularly in fibroblasts (bar = 0.5 μ m).

of particles alone. The animal model described by Howie et al.⁴⁴ showed bone resorption due to intra-articular particles, but the authors reported later difficulties in quantitating bone resorption because of the large variability in the

appearance around the implanted PMMA cylinders.⁴⁵ In other studies,⁴⁶⁻⁴⁸ additional factors such as micromotion may have influenced the outcome. In our study, we compared directly the effects of six different types of similarly-sized particle (0.5 to 2.0 μ m) with a range of size similar to that of wear particles found in interface membranes around total joint prostheses.^{2,49} Most experimental studies in vivo have used larger particles, especially those of UHMWPE and PMMA.^{21,22,24,31,32,34,44,47,50,51} We had difficulty in the preparation of small PMMA particles up to 2 μ m in size and therefore chose to use Zr particles only. A recent X-ray microanalysis study showed this metal to be present in particulate form in interface membranes around cemented total hip prostheses.⁵² Preparation of UHMWPE particles in directly phagocytosable sizes is difficult⁵³ and therefore HDPE particles have been used in many experiments because they are much easier to obtain. Differences in cellular responses to particulate HDPE or UHMWPE may exist. Finally, by using latex particles, we tried to establish whether the presence of particles induced cellular responses, regardless of the surface structure or chemical composition. The particle concentrations (10^9 to 10^{10} particles per ml) of the suspensions were at least one order of magnitude higher than that used in the bone-chamber studies of Good-

Fig. 4a

Fig. 4b

Fig. 4c

Sections of rat knees after immunohistochemical staining with ED7 primary antibody showing macrophages, granulocytes and natural killer cells (stained brown). Figure 4a – Joint during acute phase of inflammation after antigen-induced arthritis.^{39,40} Figure 4b – Synovium containing Co-Cr particles (black). In the deeper layer of the synovium, the number of macrophages stained positively with ED7 antibody is comparatively low. The macrophages in the synovial lining are stained as a positive control. Figure 4c – Synovium containing Ti-particles (black) mainly intracellularly in fibroblast-like cells (arrows) which are not stained with ED7 antibody. Most ED7 positive macrophages do not contain particles (s, synovium; ca, cartilage; bar = 100 μ m).

Fig. 5a

Fig. 5b

Fig. 5c

Photomicrographs of sections of the intramedullary canal of the femur showing the effects of isopropanol (a), Ti (b) and UHMWPE (c) particles on bone formation around PMMA implants (p, PMMA implant; c, cortical bone; arrowheads, accumulations of particles) (Giemsa; bar = 100 μ m).

man et al^{54,55} which was considered sufficient for inducing cellular responses.

To evaluate the effects of different wear particles in a vital bone environment, the model of Howie et al⁴⁴ was selected at first. After repeated injections of particle suspensions into the knees none of the specimens showed migration of particles from the joint space along the PMMA cylinder to the intramedullary space. The PMMA cylinder was completely surrounded by new bone without any evidence of osteoclast or macrophage activity. This was the reason for changing the model as described before to allow analysis of the effects of wear particles on bone.

In our study, no distinct differences in the response to the various types of intra-articular particle were observed. Thickening of the synovial membrane was the only exception, especially when UHMWPE or HDPE particles were present (Fig. 2). Our findings agree with those of Rae²⁹ in regard to the limited effects of Ti-alloy particles (<5 μ m). Howie and Vernon-Roberts^{25,26} reported intense macrophage infiltration and necrosis of these cells after injection of Co-Cr alloy particles with a mean size of 3 μ m into rat knees. Only a difference in the composition of the two Co-Cr alloys could explain the variation in the results.

In the intramedullary compartment, macrophage recruitment, the presence of giant cells and osteoclast activity or

evidence of bone resorption were not observed, irrespective of the type of particle used. Formation of new bone was impaired only in the presence of UHMWPE and HDPE particles. These findings agree with those of Aspenberg and Herbertsson⁵⁶ who reported no bone resorption at a stable and vital Ti-bone interface in rats in the presence of HDPE particles of 4 μ m in diameter. When sliding movements were applied between Ti and bone, bone resorption and

Table I. Histomorphometric data of mean number ($\pm$ sd) of osteoclasts and mean percentage ($\pm$ sd) of formation of new bone around PMMA implants in the intramedullary canal and the effects of different types of particle

Type of suspension	Number of osteoclasts*	Bone formation
0.9% saline	0.67 $\pm$ 0.98	50.5 $\pm$ 2.9
Isopropanol	1.67 $\pm$ 1.30	46.4 $\pm$ 7.3
UHMWPE	0.42 $\pm$ 0.51	13.9 $\pm$ 6.9†
HDPE	0.33 $\pm$ 0.49	11.5 $\pm$ 22.1†
Co-Cr	1.33 $\pm$ 1.97	48.4 $\pm$ 10.4
Ti	0.08 $\pm$ 0.29	46.8 $\pm$ 27.0
Zirconium	0.17 $\pm$ 0.39	53.3 $\pm$ 6.9
Latex	0.67 $\pm$ 0.58	58.7 $\pm$ 6.9

* mean total number per area of approximately 3 mm² in each section

† significantly different from controls, $p < 0.01$

formation of fibrous tissue always occurred, but were not influenced by the presence or absence of HDPE particles. After cessation of movement in the absence of HDPE particles, fibrous tissue was replaced by bone, which again did not occur in the presence of HDPE particles. Reduction of bone formation due to the presence of various particles (Co-Cr, Ti and HDPE of size approximately 3 to 5 μm) has also been observed in titanium bone-chamber studies in rabbits.⁵⁵⁻⁵⁷ Foreign-body reactions and chronic inflammatory responses were evoked which were especially florid in the presence of Co-Cr particles. This could have been caused by toxic ions in the restricted environment of the titanium chamber. Such responses did not occur in our study, in that of Aspenberg and Herbertsson⁵⁶ or in a previous study of Goodman et al.³

It has been concluded, on the basis of in vitro studies of fibroblast responses to Ti particles, that fibroblasts stimulated by particles can suppress osteoblast function which could lead to extensive bone loss by impairment of the bone-repairing processes.¹⁸ Such a mechanism could, in part, explain the observation of the reduced bone formation in the presence of UHMWPE and HDPE, as many of the particles were found to be ingested by fibroblasts.

We have demonstrated that the presence of wear particles alone is not sufficient to initiate bone resorption in vivo. Mechanical factors such as micromotion between prosthetic components and bone or high joint fluid pressure reaching the bone could be important in the process.⁵⁸ The presence of UHMWPE particles could then inhibit restoration of bone loss and enhance progressive loosening.

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