THE EFFECTS OF PARTICULATE POLYETHYLENE AT A WEIGHT-BEARING BONE-IMPLANT INTERFACE

A STUDY IN RATS

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In ten male rats we inserted ceramic 'drawing-pin' implants in weight-bearing positions within the right proximal tibia. Two animals were killed 6 weeks after surgery and two more 14 weeks after surgery. The remaining six received intra-articular injections of either high-density polyethylene (4 rats) or saline (2 rats) at 8, 10 and 12 weeks after surgery. These animals were killed two weeks after the last injection.

Histological examination of the bone-implant interface in the control animals showed appositional bone growth around the implant at both 6 and 14 weeks. Polyethylene, but not saline, caused a chronic inflammatory response with numerous foreign-body giant cells in periprosthetic tissues.

Our model of a stable, weight-bearing bone-implant interface provides a simple and reliable system in which to study in vivo the effects of particulate materials used in orthopaedic surgery.

J Bone Joint Surg [Br] 1996;78-B:32-7.
Received 14 March 1995; Accepted 20 April 1995

Aseptic loosening is the most common complication of total hip and knee arthroplasty and the most common cause of failure of arthroplasty (Amstutz et al 1982). Its incidence increases as follow-up times become longer (Olsson, Jernberger and Tryggö 1981). With a growing trend towards performing arthroplasty in the younger patient, the need for revision hip and knee arthroplasty can only increase.

Although the cause and pathogenesis of aseptic loosening is still contentious, some important factors have been identified; the most intriguing is the role of particulate wear debris. The biological responses to wear debris have been studied extensively over the last two decades (Willert 1977; Rushton and Rae 1984; Jasty et al 1986; Ohlin, Johnell and Lerner 1990; Thomson et al 1992; Harris 1994). There is good evidence that particles of a variety of metals, ceramics and polymers can damage human cells in vitro and may incite foreign-body reactions (Rae 1975; Howie and Vernon-Roberts 1988; Maloney et al 1993) or bone resorption in vivo (Murray and Rushton 1990; Ohlin et al 1990; Haynes et al 1993).

Most of the experimental studies have involved the injection of wear debris into healthy joints (Rushton and Rae 1984; Rae 1986; Howie et al 1988). Although their results provide valuable information about the response of healthy tissues to debris, they are less relevant in regard to aseptic loosening. To understand the relationship between wear debris and aseptic loosening, we must be able to study the effects of well-characterised particulate debris around a stable implant. Such an approach was pioneered by Howie et al (1988) who created a stable cement-bone interface by inserting a preformed polymethylmethacrylate plug into the distal femur of rats. Intra-articular injections of particulate ultra-high-molecular-weight polyethylene induced chronic inflammatory responses and bone resorption at the cement-bone interface.

This was the first clear evidence that particles could induce periprosthetic bone resorption but this animal model has two important limitations. First, the implant is inserted as a plug in the distal femur and, in time, tends to migrate proximally (as pins do in clinical practice) and to become sealed off from the joint space. Although of value in a short-term study the model is not useful for studying the effects of particles delivered over a protracted period. The second and more serious limitation is that the implant is inserted in a non-weight-bearing area to shield it from the effects of mechanical loading.

Micromotion is recognised as a key factor in the control of bone turnover (Wolff 1892; Lanyon 1990) and is undoubtedly important in aseptic loosening, since it affects the activity of periprosthetic osteoblasts and osteoclasts. It also affects the migration of wear debris around a loose prosthesis. Schmalzried, Jasty and Harris (1992) have shown that mechanical loading of a total joint prosthesis

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0301-620X/96/11080 $2.00

tends to force synovial fluid into any gaps in the interface between the implant and surrounding bone; in the early stages of aseptic loosening, this tends to enhance the passage of particulate-wear debris around the bone-implant or cement-bone interface.

Spector et al (1990) and Goodman, Magee and Fornaiser (1993) performed arthroplasties in dogs and rabbits, respectively, to study the role of inflammatory mediators in aseptic loosening. The components used in these studies, however, were precoated with bone cement and inserted so that they were mechanically loose at the time of implantation.

We have developed a new model to study the effects of wear debris around a mechanically stable, uncemented, weight-bearing implant and we have used this in a pilot study to investigate the effects of high-density polyethylene (HDP).

MATERIALS AND METHODS

We used ten male Sprague-Dawley rats with a mean body-weight of 200 to 250 g. The implants consisted of ceramic pins, 1.3 mm in diameter and 10 mm long, made from aluminium oxide (>99.7% pure). They were steam-autoclaved before use.

The particulate HDP was donated by Dr J. Davidson (Smith & Nephew-Richards, Memphis, Tennessee). The particle size distribution was determined by laser particle sizing with a Malvern MasterSizer 4 (Malvern Instruments, Malvern, UK). The mean particle size was 2.03 μm.

The HDP particles were sterilised in ethylene oxide before use. For intra-articular injections, a suspension of particles in sterile saline was prepared by suspending 5 mg of sterile HDP (approximately 5×10^{11} particles) in 15 ml of a 1:50 mixture of Sprague-Dawley serum in sterile phosphate-buffered saline (PBS); the final suspension contained 3×10^{10} particles/ml. The sterile serum-saline solution was used as the control for the injection of HDP particles.

Operative techniques. The animals were anaesthetised with an intraperitoneal injection of a 1:1:2 mixture of fentanyl-fluanisone (Hypnorm; Janssen Animal Health, Wantage, UK) and midazolam (Hypnovel; Roche, Welwyn Garden City, UK) in sterile water. The mixture was injected at a dose rate of 2.7 ml/kg body-weight (equivalent to 0.2 mg fentanyl, 6.75 mg fluanisone and 1.35 mg midazolam/kg/body-weight). This was sufficient to provide surgical anaesthesia for 30 to 45 minutes.

The right hindlimb was clipped, draped and prepared for surgery. A 1 cm linear skin incision was made slightly lateral to the patella and the stifle joint opened through a lateral parapatellar arthrotomy. The patella was displaced medially to allow visualisation of the tibial plateau. A drill hole 1.2 mm in diameter was made to a depth of 1 cm in the proximal tibia using a standard hand drill. A counterbore was then used to remove a full-thickness osteochondral cylinder of 0.5 mm diameter from the tibial plateau. The aluminium oxide implant was inserted in a press-fit fashion into the tibia, with the pin head lying flush with its articular surface. Bone fragments were removed from the joint by careful irrigation with sterile saline, the patella was replaced in the trochlear groove and the arthrotomy wound closed with three simple interrupted sutures of 6/0 monofilament nylon (Ethilon; Ethicon, Edinburgh, UK). The skin incision was closed with three simple interrupted sutures of 6/0 polyglactin 910 (Vicryl, Ethicon).

The implants were inserted unilaterally (right stifle joint) in all animals. Antibiotics (Synulox; Beecham, Brentford, UK) and analgesics (Temgesic; Reckitt and Coleman, Hull, UK) were given routinely in the immediate postoperative period.

Injection of particulate materials. The animals were anaesthetised and both stifle joints clipped and prepared for surgery. Intra-articular injections were made with a 26G hypodermic needle by a transpatellar approach. At 8, 10 and 12 weeks after surgery we injected 100 μl of either HDP (equivalent to a dose of 3×10^9 particles) or the saline control into both the operated and the contralateral (unoperated) stifle joint. The animals were killed two weeks after the final injection (14 weeks after surgery).

Control animals. Four rats had a prosthesis implanted but did not go on to receive intra-articular injections. Two were killed at 6 weeks and the remaining two at 14 weeks after surgery.

Postmortem examination. We performed full postmortem examinations on all the animals. Samples were removed from any organ showing gross evidence of pathology. Both hind legs were removed *en bloc* and fixed in neutral-buffered formal saline. The femur and tibia were disarticulated by cutting all soft tissues connecting the two bones.

Radiography. High-definition craniocaudal radiographs of the right tibia were obtained to assess placement of the implant.

Histopathology. Tibiae containing the ceramic implant were dehydrated in ethanol and embedded in plastic (Norpol 35-50; Bondaglass Voss, Beckenham, UK). A low-speed diamond saw (Accutom; Struers, Glasgow, UK) was used to prepare 500 μm-thick transverse sections of undecalcified bone at four levels along the length of the pin (Fig. 1). Sections were glued on to perspex slides and surface-polished with a graded series of silicon carbide polishing discs on a variable-speed polishing wheel (DAP-7, Struers). They were then surface-stained with 0.25% toluidine blue and examined under a light microscope (Dialux 20; Leica, Milton Keynes, UK) equipped with a polarising lens.

RESULTS

The animals recovered well after pin implantation and were weight-bearing on the operated leg within 72 hours of surgery. They recovered from the effects of the intra-

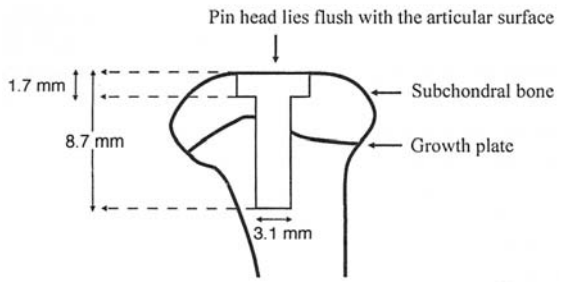

Fig. 1

Schematic diagram of the proximal rat tibia to show the location of the histological sections prepared at four levels of the bone-implant interface.

articular injections within 24 hours.

Postmortem findings. All the surgical wounds had healed well. Monofilament nylon sutures were still present in the joint capsule of the operated stifle joints but there was no evidence of adverse tissue reactions around these sutures. The head of each implant was clearly visible in the tibial plateau.

Radiography. Correct placement of the implant was confirmed by radiography. The implant head was flush with the articular surface so that the pin was loaded when the animal walked.

Histopathology of the bone-implant interface. Six weeks after surgery there was appositional bone growth up to and around the prosthesis at all levels below the head of the pin which was surrounded by articular cartilage of normal appearance. At 14 weeks after surgery the amount of new bone around the implant had increased and there was evidence of active bone remodelling.

The histological response in the saline-injected animals was similar to that of the non-injected control group. There was a small amount of fibrous tissue around the head of the pin at the interface between the implant and the surrounding cartilage. Below the head of the pin, new bone formed a thin shell around the implant and there was no evidence of an inflammatory reaction (Fig. 2).

The bone-implant interface in animals which had been injected with particulate HDP appeared abnormal. Under low-power microscopic examination, areas of periprosthetic bone loss, fibrosis and chronic inflammation were seen. These resembled large cystic cavities filled with mononuclear cell infiltrates and fibrous tissue (Fig. 3a). Clusters of multinucleate giant cells were present within the fibrous tissue (Fig. 3b) and under high power they were seen to be grouped around collections of amorphous, brown-staining particulate debris (Fig. 3c). The particles did not show birefringence under polarised light, although this was almost certainly because of the thickness of the sections (500 μm). Particles (and giant cells) were common in sections cut from the proximal tibia (level 2) but were not seen in sections cut through the distal pin.

DISCUSSION

Particulate wear debris is strongly implicated in the pathogenesis of aseptic loosening of arthroplasty components. We have developed an animal model on which the effects of particulate debris may be tested either in isolation or in combination.

Six weeks after surgery there was abundant appositional formation of new bone around the implant which persisted

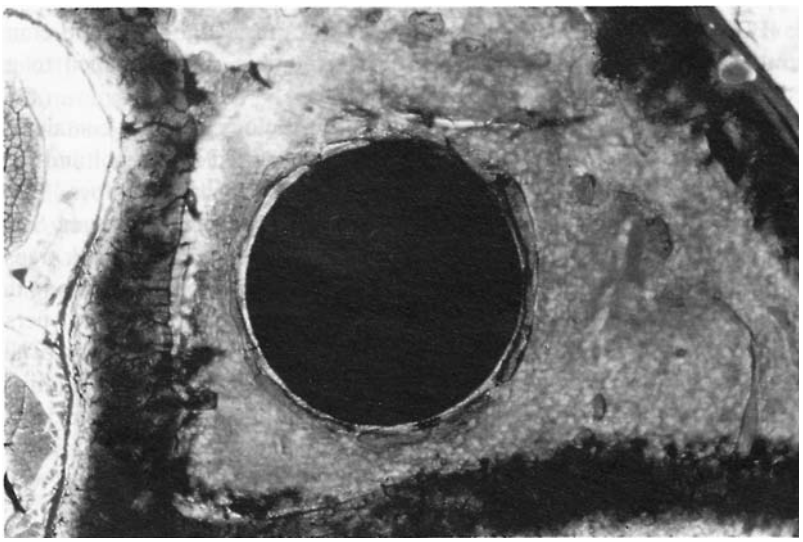

Fig. 2

The bone-implant interface in a saline-injected animal killed 14 weeks after surgery. New bone surrounds the implant and there is a thin fibrous layer between the implant and the surrounding bone, but no evidence of inflammation (toluidine blue × 18).

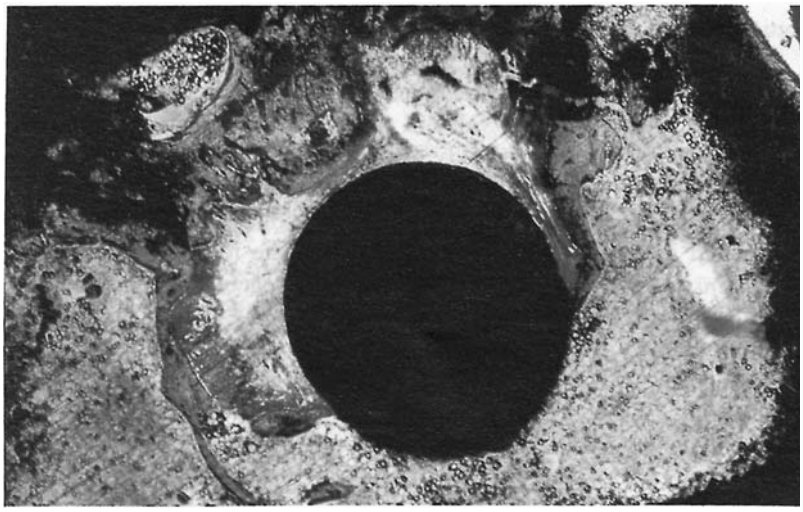

Fig. 3a

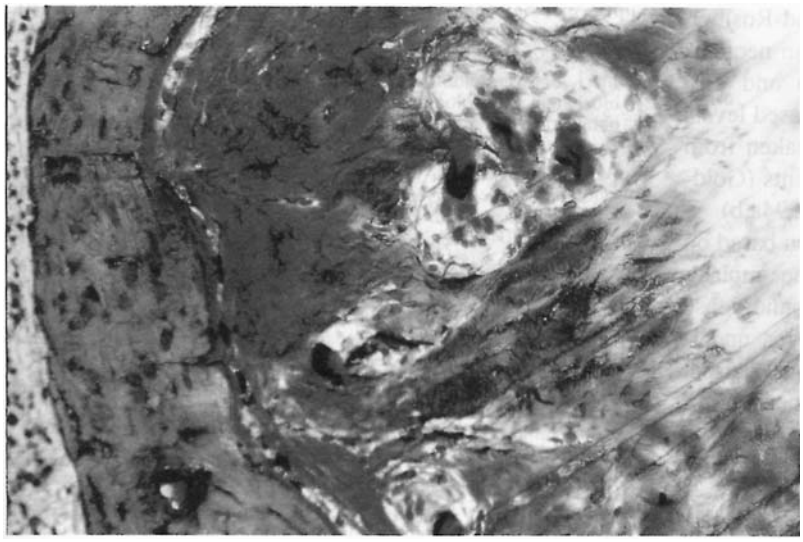

Fig. 3b

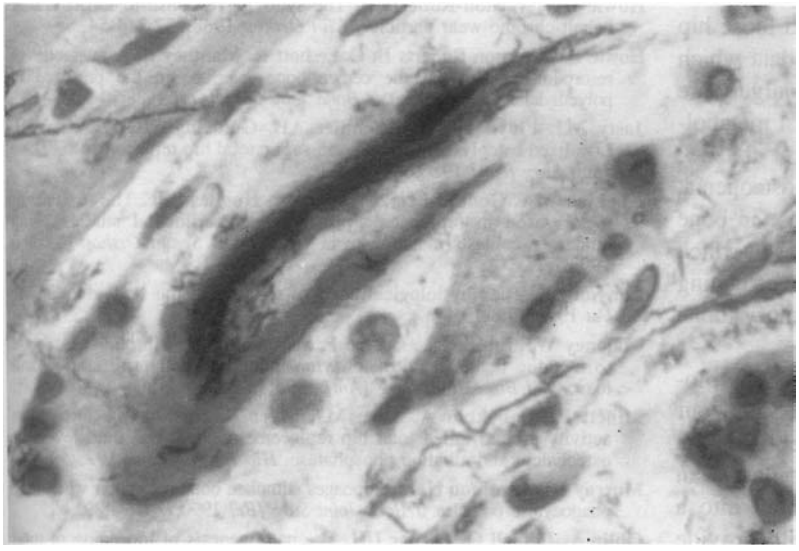

Fig. 3c

The bone-implant interface from an animal which received three intra-articular injections of HDP. Periprosthetic bone has been replaced by a lytic lesion containing fibrous tissue (a). Multinucleate giant cells are evident (b) associated with the presence of aggregates of brown-staining particulate debris (c) (toluidine blue $\times$ 18(a), $\times$ 70(b), $\times$ 270(c)).

for the entire 14-week experimental period. Injections of particulate HDP at intervals after the six-week 'healing' period caused a dramatic reduction in the amount of appositional new bone within the proximal tibia. In these regions periprosthetic bone was replaced by chronic inflammatory tissue containing both particles and giant cells. Around the lower portion of the pin there were no particles or giant cells and the periprosthetic bone appeared normal. These findings support the idea of a firm link between debris, inflammatory responses and periprosthetic bone loss.

The effects of particulate wear debris on periprosthetic tissues have been assumed to be related to a net increase in bone resorption. This hypothesis is supported by considerable experimental data relating to the stimulation of bone-resorbing activity from cells exposed to particulate materials in vitro. A number of bone-resorbing factors have been identified in these studies, including interleukin-1 (Glant et al 1993), prostaglandin E_2 (Murray and Rushton 1990; Glant et al 1993; Haynes et al 1993), tumour necrosis factor and interleukin-6 (Haynes et al 1993) and collagenase (Maloney et al 1993). In addition, increased levels of these mediators have been found in tissues taken from around loose hip and knee arthroplasty components (Goldring et al 1983; Appel et al 1990; Chiba et al 1994a,b).

Since the diagnosis of implant loosening is often based on the identification of osteolytic lesions around the implant, this widespread interest in bone resorption around loose implants is logical. Periprosthetic bone is, however, undergoing constant turnover and the amount of bone present around the implant is determined by the dynamic equilibrium that exists between bone resorption and the formation of new bone. We believe that in addition to a direct effect on bone resorption, wear debris may influence periprosthetic bone turnover by an inhibitory effect on bone formation. Preliminary evidence from our own laboratory has shown that the levels of osteocalcin and alkaline phosphatase are reduced in samples of synovial fluid from around loose hip replacements (Millett et al 1995). We now have data which prove that particulate metals used in orthopaedic surgery are capable of causing cytotoxicity and an inhibition of metabolic activity in osteoblastic cells in vitro (Khokher et al 1993; Allen et al 1995). We believe that, as in disuse osteopenia, reduced bone formation combines with an increase in bone resorption to cause the alterations in bone turnover which precede implant loosening. We are at present investigating this further using fluorochrome labels to quantify bone turnover around the pin implant.

Our rat model will also allow us to study the biocompatibility of particulate materials in vivo. Since our main aim is to study the effects of biomaterial debris on the joints of patients who have undergone arthroplasty, an animal model in which test materials are injected into a joint containing a weight-bearing implant will provide more relevant information than one in which debris is injected into a healthy joint. Tissues which have undergone surgery may respond differently to normal tissues, a factor which may be important in the pathogenesis of inflammatory and/or neoplastic lesions around orthopaedic implants.

Our results validate the model as an appropriate test system for the study of the relationship between a weight-bearing implant and particulate wear debris. We now plan to use this animal model to investigate a range of metals, ceramics and polymers in particulate form to improve our understanding of the effects of wear debris on periprosthetic bone.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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