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An In Vivo Murine Model of Continuous Intramedullary Infusion of Polyethylene Particles

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INTRODUCTION

Total joint replacement (TJR) has been the most effective surgical procedure for restoring function and mobility to millions of patients with osteoarthritis and rheumatoid arthritis[1]. However, the longevity of total joint replacements is often jeopardized by wear debris associated aseptic loosening and osteolysis [2–4]. Understanding the mechanisms of chronic inflammation and periprosthetic osteolysis, and developing prostheses with increased longevity have become paramount within the orthopaedic community.

Immediately after implanting a TJR, the bone-prosthesis interface is exposed to continuously generated wear debris over time. Wear of the UHMWPE component accounts for the majority of the wear particles generated in initially well-fixed prostheses[5]. The penetration rate of the head into the polyethylene acetabular cup has a linear relationship with the occurrence of osteolysis 10 years following implantation[6]. Furthermore, analysis of retrieved implants showed that the concentration of wear debris particles is directly related to the duration of implantation, and that osteolysis is usually present when the number of particles exceeds 1×10^{10} /g of tissue[7]. Analysis of retrieved tissue from early stage and late stage THRs suggested that the accumulation of wear debris over time lead to the osteolysis[8].

UHMWPE particles isolated from retrieved tissue from failed THR vary greatly in size and morphology. However, the majority of the particles are globular spheroids that are 0.1–0.5um in diameter [9–11]. These particles stimulate the differentiation, maturation and activation of osteoclasts, interfere with osteoblast function and eventually disturb the normal homeostatic balance between bone formation and degradation [12,13]. Studies of the response of macrophages to clinically relevant particles have shown that particles with a mean size of 0.24 um stimulated bone resorption at a ratio of 10 um^3 /cell, whereas particles of 0.45 and 1.71 um were active at a ratio of 100 um^3 /cell [1].

Different animal models have attempted to simulate the biological processes of particle associated osteolysis in vivo [14–19]. To date, none of the studies was performed on a murine model incorporating the presence of a stable intramedullary implant and the continuous delivery of particles. Studies of clinically relevant polyethylene wear particles are rare because of the limited availability of UHMWPE particles and their low density causing the particles to float.

In this study, an in vivo murine model integrating continuous infusion of clinically relevant polyethylene particles and an implanted hollow titanium rod is elucidated using the technique of uCT and histology.

MATERIALS AND METHODS

Animals

Adult, 9 week old, male, C57BL/6 wild type mice were obtained from the university in-house breeding colony. Animals were divided into two groups randomly. One group of animals (N=13) had a right hollow distal femoral rod and infusion of carrier solution alone without particles, and a left non-operated limb. The other group of animals (N=10) received bilateral hollow distal femoral rod implants. In these 10 mice, UHMWPE particles in carrier solution were infused over a 4-week period using an infusion pump and tubing in the right femur only (Table 1). This experimental design was adopted because of the difficulty of infusion of fluid bilaterally, which would necessitate two Alzet pumps in one animal.

Surgery

General anesthesia was induced by 3% isoflurane in 100% Oxygen. Using sterile technique, the intercondylar notch of the distal femur was exposed through a medial parapatellar arthrotomy. A 23-gauge needle was used to manually drill through the intercondylar notch to access the medullary cavity. A 23-gauge hollow rod, 6-mm long, was press fit into the distal femur. When indicated, the rod was connected to polyvinyl tubing and an Alzet pump (Model 2004, Durect Corporation, Cupertino CA) implanted subcutaneously between the scapulae through a separate incision. After implant insertion, the quadriceps-patellar complex was repositioned and the medial quadriceps arthrotomy and dorsal incision for the pump were repaired with 5.0 vicryl sutures. Euthanasia was carried out in a CO₂ chamber 4 weeks after surgery.

Particles and pumps

Conventional UHMWPE particles, a generous gift from Dr. Tim Wright at the Hospital for Special Surgery at New York were obtained from knee joint simulator tests and isolated according to an established protocol [9]. The mean diameter of the particles was $0.49 \mu\text{m} \pm 0.28 \mu\text{m}$ (mean $\pm$ standard deviation, determined by electron microscopy). The Limulus Amoebocyte Lysate assay (BioWhittaker, Walkersville, MD) was performed to ensure that the particles were endotoxin free. Approximately 1.5×10^{11} particles were reconstituted in mouse serum (the carrier) and loaded into each pump (0.25 μl /hour delivery rate, Durect, Cupertino, CA) for infusion. The mean volume of the pumps was 246 μl and the mean pumping rate was 0.2 μl / hr according to the manufacturer.

Microcomputed tomography analysis

After sacrifice, a detailed qualitative and quantitative 3-D evaluation was performed of the distal 2/5 of each femur using a Scanco Viva40 microCT scanner with 10 micrometer resolution. A fixed threshold was applied to assess mineralized bone on the grey scale images. The bone volume divided by total volume (BV/TV) was used for statistical analysis.

Histology and histomorphometry

Following decalcification, each femur was cut into three pieces, starting from the distal end of the femur and moving proximally for each measured cut (Figure 1). The remaining portion of the most proximal end of the femur was discarded. Each of the three cut pieces of femur was embedded in paraffin, sectioned into 6 μm slices and mounted on glass slides. This process was performed as specified so that samples of bone could be examined histologically from the

mid-rod region (Figure 1, cut #1), proximal rod region (Figure 1, cut #2) and a region of bone far proximal to the end of the rod (Figure 1, cut #3). Thus, the lines on Figure 1 have to be changed to reflect this. Cut #1 should be more toward the middle of the rod.

The cortical areas from each collected femur were measured by a computer program (Axioversion, Zeiss). The average value from the distal and middle cuts was compared for each treatment group.

Immunohistochemistry

The expression of Alkaline Phosphatase (Alk Phos) was detected by a monoclonal antibody (Cat #: AF2910, R & D systems) using the decalcified femur embedded in paraffin. The positively stained area was quantified using Image Pro software and divided by tissue section area.

The expression of F4/80, a marker for a 60 kD cell surface glycoprotein on the macrophages was detected by immunohistochemistry (Histo-tec Laboratory, Hayward, CA).

Data analysis

Paired t test and Wilcoxon signed-rank test were performed on animals to compare the left and right femurs. A p value less than 0.05 was considered significant.

RESULTS

Radiographs were taken of the animals immediately after surgery and before euthanasia to verify the position of the implants. As shown on the radiographs (Fig. 2), the titanium rod was placed within the distal 2/5 of the medullary canal, parallel to the longitudinal axis of the femur. No subsequent change in position of the rod was observed on the x-rays taken after the 4-week infusion period.

We then quantified the bone volume contained in the distal 2/5 of the femur (BV/TV) by using of micro CT scanning (Figure 3a). For animals that were infused with carrier on one side and had a contralateral non-operated femur, the relative amount of bone (BV/TV) did not differ (Figure 3b). In contrast, infusion of UHMWPE particles reduced the bone volume by 9.15% when compared to the contralateral side of the same animal in which the rod alone was implanted ($p = 0.028$) (Figure 3b).

On the H & E stained sections, normal marrow cellular constituents could be seen in all the samples. The previous location of the rod was easily identifiable. The endosteum of the cortex was mostly smooth. The cortex of the UHMWPE infused femurs appeared to be more porous than other groups (Figure 4a). Histomorphometry did not find any differences in the femora on both control animals and experimental animals.

The infusion of serum by itself led to the highest level of Alkaline Phosphatase expression when compared to non-operated femurs ($p = 0.04$). Infusion of UHMWPE did not change the level of Alkaline Phosphatase expression when compared to the contralateral femur in which only the rod was placed (Figure 4b).

Macrophages have been implicated in the process of particle associated osteolysis. Therefore, F4/80 expression, a specific marker for mature macrophages including macrophages in the bone marrow stroma was also examined. A large number of F4/80 positive macrophages / monocytes were present in all the sectioned femurs. (Figure 4c).

Operated femurs generally contained more macrophages than the non-operated ones. UHMWPE infusion did not appear to yield higher numbers of macrophages than the contralateral control side. Due to the limited sensitivity of the computer software to pick up the colorimetric signal (which not fluorescent), the total amount of F4/80 was not able to be quantified further via this method.

DISCUSSION

Several animal models have contributed to our current understanding of the biological mechanisms associated with loosening of total joint replacements. In particular, Jacobs et al. demonstrated the adverse effects of spinal implant debris utilizing a rabbit osteolysis model [20]. Bostrom et al and Kim et al studied particle induced osteolysis in a rat model [17,21], and Shanbhag et al studied the effects of particles in hip arthroplasty using a canine model [22]. Developing murine models is of particular interest because mice are hearty, and the surgical procedures are less costly than with larger animals. Furthermore, the availability of genetically manipulated variants and molecular methods makes murine models even more advantageous in identifying underlying biological mechanisms.

Utilizing an osmotic pump to deliver clinically relevant particles to a joint in rats was first described by Kim et al. Due to the technical difficulty of quantifying UHMWPE particles contained in the femur, the number of particles that actually reached the targeted destination was never known. Recently, the efficiency of the delivery system coupled with the mouse femur was carefully examined by Ortiz et al in vitro. According to these studies, around 32 % of UHMWPE (4.7×10^{10}) are successfully delivered to the bone marrow cavity after 4 weeks, if 1.5×10^{11} particles are loaded into the pump. In the present study, we loaded the same number of particles as Ortiz et al did to stay consistent with the previous findings.

Generation of UHMWPE particles has been implicated as the major factor leading to osteolysis [8,23–26]. With the present model, significant reduction of net bone volume in the UHMWPE treated group was observed via microCT scans. This finding suggested that the delivery of UHMWPE particles was associated with bone loss using this murine model. However, the same reduction of bone volume was discrepant with the histological analysis. One possibility is that the histomorphometric data was associated with higher sampling error because only one slide was assessed from the distal and the middle portion of each femur. According to the microCT data, reduction of bone volume in this model following the infusion of UHMWPE for 4 weeks was around 10%. To detect such a small difference, a high sensitive assay is critical.

It is well known that serum contains growth factors that promote stem cell proliferation and differentiation. Complete mouse serum was chosen as a vehicle in this study because it facilitated the suspension of the UHMWPE particles compared to standard buffer solutions and is related more closely to the clinical situation. The level of Alkaline Phosphatase expression was significantly higher in the femurs that were infused with complete mouse serum than the non-operated femurs, suggesting that infusion of serum by itself might have stimulated osteoprogenitor cell/osteoblast proliferation, differentiation and/or expression. Interestingly, when UHMWPE was infused with the serum together, the Alkaline Phosphatase level was no different from the sham operated femur. This data suggested that the suppressive effect of UHMWPE particles on Alkaline Phosphatase expression was as potent as the stimulatory effect of complete serum so that no difference could be detected following the combination of the two treatments.

Numerous studies have documented the importance of UHMWPE activated macrophages in the processes associated with osteolysis [12,21,27,28]. Because accurate quantification of the

positively stained areas was extremely difficult using the tissue sections, we did not quantify the macrophages. A more sensitive detection method is needed for future studies.

The present model simulates the continuous delivery of particles to a bone-implant interface, is cost-effective, and utilizes clinically relevant particles (from wear simulator studies) similar to those generated in vivo. In addition, molecular analyses of the retrieved specimens from mice are possible, which is a distinct advantage compared to the use of larger animals. Studies using this model are ongoing to further understand the complex biological processes associated with bone loss due to wear particles.

CONCLUSIONS

Continuous infusion of particles to the murine bone-implant interface simulated the clinical scenario of local polymer wear particle generation and delivery in humans, and can be used to further study the biological processes associated wear debris particles.

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Figure 1. The dissection of the harvested femur for histology

Each femur was cut into three blocks. The first cut was performed 4 mm proximal to the femoral condyles, the second cut occurred 3 mm proximal to the first cut and the third cut occurred 3 mm proximal to the second cut. The remaining proximal end of the femur was discarded. 4um tissue sections were collected from each of the three tissue blocks.

Figure 2. X ray radiograph showing the position of the rod and the particle delivery system
a. An Alza osmotic pump loaded with the particles was connected with silicone tubing overlying a 6 mm long hollow titanium rod at the end. The tubing was directed through the subcutaneous tunnel, and the titanium rod was inserted into the femur until the end of the rod was flush with the end of the femur.
b. Radiograph taken after the femur was harvested providing a closer viewer of the position of the rod.

Figure 3. μ CT scans of the harvested femur

a. Each femur was harvested after the experiment. The distal 2/5 portion of the femur was scanned by a μ CT. Sagittal view and transverse views of representative femurs are shown. Notice that the cortical areas of the UHMWPE infused femurs are more porous.

b. The bone volume (BV) contained in the total volume (TV) of the femur being scanned is plotted. Infusion of UHMWPE particles led to decreased BV/TV when compared the

contralateral side where only a rod was placed. Infusion of serum did not change the BV/TV when compared to the non-operated contralateral side.

Figure 4. Histology and immunohistochemistry of the harvested femurs

Each femur was decalcified, cut into three blocks, and embedded in paraffin. 4 μ m sections were obtained from the distal, middle and proximal portion of the femur.

a. Classical H & E staining was performed on all the sections. The outline of the rod previously present was clearly visible. Bone marrow cells appeared normal. New bone formation around the implant can be observed.

b. Immunohistochemistry of Alkaline Phosphatase (Alk Phosp) expression. Infusion of serum led to significantly increased AlkPhos expression. Within the non-operated femurs, Alk. Phos expression can only be seen sparsely. No difference was found between the UHMWPE infused group and the contralateral side when only a rod was placed.

c. Immunohistochemistry of F4/80 expression. Macrophages were detected by the expression of F4/80 on mature macrophages. Abundant macrophages were observed in every group.

Table 1
Experimental Design