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The inhibition of neutrophil antibacterial activity by ultra-high molecular weight polyethylene particles

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Abstract

Following infection, bacterial killing by polymorphonuclear leukocytes (neutrophils) is the main host defense against bacteria. Our hypothesis is that particles of ultra-high molecular weight polyethylene (UHMWP) may impair local neutrophil function and consequently reduce neutrophil bacterial killing. To determine how the in vitro phagocytic–bactericidal activity of neutrophils was affected by exposure to wear particles, tests were run comparing the effects of different particle composition, and different concentrations and sizes of UHMWP particles. There was a significant correlation between the number of particles and the decrease in neutrophil bactericidal activity ($p<0.01$), and the greatest effect was obtained with a concentration of 10^7 UHMWP/ml. There was a significant decrease in neutrophil bactericidal activity by incubation with particles of 0.1–5 μm ($p<0.01$), but not with larger size. The results suggest that neutrophil functional defects triggered by the presence of UHMWP particles may potentially contribute to the susceptibility of loose implants to bacterial infections.

Keywords: Neutrophil; Antibacterial activity; Polyethylene particles

1. Introduction

The two major complications following total joint arthroplasty are osteolysis with resultant component loosening, and microbial infection. The pathogenic mechanisms in both instances are not fully elucidated. Joint prostheses annually produce billions of submicrometer wear particles of ultra-high molecular weight polyethylene (UHMWP) that can result in osteolysis and component loosening. The chronic inflammatory response to particle wear debris has been well-documented [1]. With regard to infection, bacterial killing by polymorphonuclear leukocytes (neutrophils) represent a major host defense against microbial pathogens. The susceptibility of implanted biomaterials to bacterial infections results from the combined contribution of microbial factors, such as adhesins and biofilm formation, and interfacial reactions with host defense mechanisms. Several recent reports have described the impact of anaerobic bacterial biofilm formation in hip joint infection and the resultant increase in antimicrobial activity [2]. High concentrations of wear particles in joint fluids may potentially lead to significant inhibition of phagocytosis and bacterial killing by neutrophils and other phagocytic cells [3].

Studies have shown that either neutrophils or macrophages can phagocytize small fragments of biodegraded or corroded metallic or plastic implants, and such particles may persist intracellularly for a prolonged

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period of time [4]. The composition of the particles themselves has also been shown to affect cellular response. On the contrary, the presence of large-size, nonphagocytosable particles may dampen the bactericidal activity of phagocytes by the so-called reaction of "frustrated phagocytosis" [5].

The objective of our study was to develop an in vitro experimental system for exploring mechanisms of interaction between orthopedically relevant microbial organisms and various types of phagocytic and nonphagocytic cells exposed to polyethylene or metallic wear debris. We selected human neutrophils and *Staphylococcus aureus* for these pilot studies, to evaluate how UHMWP released from prosthetic components impaired the microbicidal activity of neutrophils. Using this in vitro model, we first compared the effects of different sizes and concentrations of polyethylene on *S. aureus* killing by neutrophils. Second, we compared the effects of wear particles released from different prosthetic materials, namely UHMWP, commercially pure medium molecular weight polyethylene (MMWP) particles, and commercial latex (polystyrene) particles, with respect to their inhibition of *S. aureus* killing by neutrophils.

2. Materials and methods

2.1. Preparation of polyethylene particles

2.1.1. In vitro generation of wear debris from a prosthetic material

An acetabular component of a total hip prosthesis (Protek AG, Sulzer medica, Bern, Switzerland) was rasped at slow speed (1500 rpm) for 5 days with a Metabo® machine (Gs E7141, Metabowerke Gmb&Co, Nürtingen, Germany). The powder produced from rasping was ground slowly (1500 rpm) over 24 h inside a closed sterile recipient, and the wear particles were collected aseptically in water with albumin (0.01%). To fractionate the collected UHMWP debris with respect to their specific sizes, the particles were vortexed, ultrasonicated for 30 min, and then allowed to sediment for 2 h. Sedimentation was performed to separate the larger debris fraction from the finer particles that remained in suspension. The different supernatant fluids were pooled, and vortexing and sedimentation were repeated several times. To prevent clogging of filter membranes, the suspension of fine particles was sequentially filtered through pre-weighed polycarbonate membranes (Whatman Limited, Maidstone, Kent, UK) with pore sizes of 50, 10, 5, and 0.1 μm. For the commercially pure MMWP particles and commercial latex particles, a similar technique was used to obtain a final concentration of 5×10^7/ml.

2.1.2. Preparation of wear debris from a prosthesis in vivo

In three patients, periprosthetic neo-capsular tissue was removed during revision total hip arthroplasty (Protek, Sulzer medica, Bern, Switzerland). UHMWP particles were extracted from each tissue using a previously described and validated method. In brief, at the time of resection, the tissue was immediatly immersed in neutral buffered formalin and stored at 20 °C. Particles of wear debris were isolated from the tissue by sodium hypochlorite digestion. At the end of digestion, the extracted particles were analyzed and compared to particles using scanning electron micrograph [6].

2.1.3. Analysis of wear debris with scanning electron microscopy

Particle suspensions were placed over polycarbonate filters, allowed to dry, and gold sputter-coated (Balzers, SCD 030, Liechtenstein). The in vivo and in vitro particles were analyzed with scanning electron microscopy (SEM; Philips XL-20 FEG Mahwah, NJ). For size analysis, two orthogonal diameters were measured from the SEM images, to evaluate the median size of each particle. Frequency distributions and cumulative percentages of dimensions were based on estimates from 50 particles. Histograms were prepared to represent size distribution.

2.1.4. Sterilization of wear debris

Particles were sterilized five times by irradiation using a total dose of 0.6 J/cm^2 (SpectroLinker, Spectronics corporation, AMS technology). Sterilization was verified by culturing each sample preparation in Mueller–Hinton (MH) agar.

2.2. Preparation of neutrophils

Neutrophils were purified from heparinized blood using the Boyum's method [7]. After dextran sedimentation, hypotonic lysis, and Ficoll-hypaque centrifugation, the neutrophils were washed twice, then re-suspended in phosphate-buffered solution (PBS) supplemented with glucose (5%), and finally kept on ice until used. The number of cells was determined using a Coulter counter (Coulter Electronics Ltd., Dunstable, UK). The final concentration of neutrophils was adjusted to 5×10^6/ml.

2.3. Preparation of bacteria

S. aureus (Cowan I ATCC 12598) was grown overnight at 37 °C in 1 ml of MH broth (Sigma). The following day, the bacteria were centrifuged (2000*g* for 15 min), washed, and re-suspended in PBS to a final volume of 1 ml. The optical density of the bacterial suspension was measured at 620 nm (U-2000 double beam spectrophotometer, Hitachi, Ltd., Tokyo, Japan).