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The Role of Oxidative Stress in Aseptic Loosening of Total Hip Arthroplasties

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ABSTRACT

This study investigated the hypothesis that wear particle-induced oxidative stress initiates osteolysis after total hip arthroplasty (THA). Patient radiographs were scored for osteolysis and periprosthetic tissues were immunostained and imaged to quantify polyethylene wear, inflammation, and five osteoinflammatory and oxidative stress-responsive factors. These included high mobility group protein-B1 (HMGB1), cyclooxygenase-2 (COX2), inducible nitric oxide synthase (iNOS), 4-hydroxynonenal (4-HNE), and nitrotyrosine (NT). The results show wear debris correlated with inflammation, 4-HNE, NT and HMGB1, whereas inflammation only correlated with NT and HMGB1. Similar to wear debris and inflammation, osteolysis correlated with HMGB1. Additionally, osteolysis correlated with COX2 and 4-HNE, but not iNOS or NT. Understanding the involvement of oxidative stress in wear-induced osteolysis will help identify diagnostic biomarkers and therapeutic targets to prevent osteolysis after THA.

Total joint arthroplasty (TJA) is the standard of care for advanced degenerative joint disease in the United States, with over 600,000 total hip (THAs) and total knee arthroplasties (TKAs) being performed each year [1]. It is projected that the number of annual TJA surgeries will exceed 4,000,000 by the year 2030. Although complications after joint arthroplasty are relatively low, approximately 10%–20% of all TJA surgeries result in additional surgeries, which require implant arthroplasty or other medical interventions to restore mobility. The foremost complication limiting implant longevity in the United States is aseptic loosening due to polyethylene (PE) wear debris-initiated chronic inflammation and inflammatory-mediated bone resorption [2–6]. Progressive bone loss at the bone–implant interface results in implant loosening, instability, and ultimately revision surgery. Due to advanced age and the loss of surrounding bone, revision surgeries have poorer outcomes. Thus, early diagnosis and treatment of osteolysis to reduce the number of revision surgeries would significantly improve patient quality of life and reduce the economic burden. Currently, there is no specific diagnostic marker for the identification of early osteolysis in THA patients, nor is there a treatment to prevent osteolysis.

The generation of implant wear debris from the articulation of metal on PE components is known to affect the activation and senescence of resident cells including macrophages, fibroblasts, osteoclasts, and osteoblasts [2,7–13]. Activation of both resident and recruited macrophages following ingestion of biologically-indestructible PE wear particles results in the production and release of pro-inflammatory cytokines, chemokines, [2,13,14] reactive oxygen species (ROS) [15], and reactive nitrogen species (RNS) [16–18]. These products do little to remove the debris, but inadvertently affect the activity, proliferation, differentiation and apoptotic responses of osteoclasts and osteoblasts. Additionally, resident macrophages have the potential to differentiate into fully functional osteoclasts in response to wear debris-mediated inflammation [7]. Thus, the chronic inflammatory cascade induced by PE wear debris ultimately leads to enhanced bone resorption and the development of osteolysis.

Bone resorption is controlled by a system comprised of three key proteins, RANK (receptor-activator of nuclear factor kappa beta), its ligand RANKL (receptor-activator of nuclear factor kappa beta ligand) and a decoy receptor OPG (osteoprotegerin). Many inflammatory cytokines (e.g. interleukin-1β, tumor necrosis factor-α, prostaglandin E2 (PGE2)) increase the RANKL/OPG ratio and/or have direct effects on osteoclastogenesis and bone resorption [12,19]. Like RANKL, these factors induce the production of ROS by NADPH-oxidase (NOX), which are required for the differentiation and activation of osteoclasts [20–26]. Thus, ROS and ROS-induced oxidative stress play a major role in regulating osteoclast function and bone resorption.

Despite the importance of ROS in osteoclastogenesis, a limited number of studies have focused on the involvement of oxidative stress in aseptic loosening. A single study suggested that overproduction or inadequate removal of ROS may be involved in the formation of fibrotic pseudocapsular tissues around revised THA components [27]. Indeed, oxidative stress is known to participate in the development of fibrosis associated with TKA [28]. Both phagocytosis and pro-

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inflammatory cytokines initiate macrophage generation of ROS by NOX and nitric oxide (NO) by iNOS [15]. More importantly, as systemic oxidative stress is associated with age-related loss of bone mass [29] and ROS is known to drive osteoclastogenesis and bone resorption [20–23,30], the combination of the two most certainly factors into post-THA osteolysis.

RNS is also likely to be involved in the development of post-THA osteolysis, as it is generated by the simultaneous production of NO and ROS (e.g. superoxide anion) by resident macrophages, osteoclasts, or fibroblasts. Detection of RNS in tissues is determined by measuring NT accumulation. Three studies have suggested that iNOS and RNS production plays a role in aseptic loosening [16–18]. Suh, Chang et al reported a significant correlated increase in iNOS expression and NT accumulation in periprosthetic tissue compared to primary surgical control tissues [18]. The levels of both were higher in non-cemented THA tissues compared to cemented THA tissues, but the increases did not correlate with the extent of wear debris-induced osteolysis for either cemented or non-cemented THA. Similarly, Puskas, Menke et al found significant differences in the amount of iNOS protein and NT accumulation in tissues from loose osteolytic and loose non-osteolytic THA compared to primary surgical controls, but no significant difference between loose osteolytic and loose non-osteolytic tissue levels [16]. Contrary to these studies, Stea, Visentin et al reported that iNOS protein increased proportionally with the extent of osteolysis [17]. In their study, tissues were collected not only from metal on PE implants, some of which were cemented, but also ceramic on ceramic THAs. Of the three studies, only Stea, Visentin et al specifically looked at the amounts of iNOS and NT in regards to the degree of osteolysis.

Based on the hypothesis that oxidative stress mediates wear particle-induced bone resorption and osteolysis, we focused on five specific osteoinflammatory and oxidative stress responsive factors. The first two potential serum diagnostic factors were HMGB1, a cytokine and alarmin released from macrophages, dendritic cells, and osteoblasts, which regulates RANKL-induced osteoclastogenesis [31], and 4-HNE, an oxidized lipid product that accumulates during age-related bone loss [29]. COX2, an ROS-producing enzyme, was included based on the ability of this enzyme to generate 4-HNE and a previous study showing it played a role in aseptic loosening [32]. iNOS and its RNS product NT were included based on their potential role in wear debris-mediated inflammation and osteolysis [17,18]. The objectives of this study were to 1) quantify the levels of five oxidative stress markers, three that play a direct role in osteoclastogenesis or bone resorption (COX2, HMGB1, iNOS) and two products that are elevated in conjunction with the loss of bone mass (NT and 4-HNE), and 2) correlate the amounts of these markers with the presence of wear debris, inflammation and the degree of THA osteolysis. Looking at the levels of these markers based on the severity of osteolysis provides insight into a potential role for oxidative stress as a mediator of both the onset and progression of THA osteolysis, and that inhibitors of oxidative stress may slow wear debris-associated osteolysis.

Methods

Tissue Collection and Patient Clinical Information

Hip tissue specimens from regions adjacent to the implanted device were obtained from 18 THA patients at the time of revision surgery. All tissue specimens were collected by surgeons at the Rothman Institute, Thomas Jefferson University Hospital, Philadelphia, PA. All identifying information was removed, and the tissues were processed according to the IRB guidelines at Drexel University. Inclusion in this study was based on patient consent to participate and implant type. To standardize the type of bearing surface material, the patient had to have received a conventional, gamma-air sterilized THA polyethylene component. Exclusion criteria included revision for infection, a previous revision surgery, or a cemented implant. The patients were placed into four groups based on the degree of osteolysis (Table 1). Based on radiographic scoring the severe osteolysis (>2 mm) cohort included six patients (implantation time 11.5–19.8 years; average 16.0 years). The moderate osteolysis (<2 mm) cohort included four patients (10.8–25.0 years; 16.7 years). The mild osteolysis cohort based on intraoperative observation and not visible radiographic osteolysis included seven patients (5.1–20.2 years; 16.0 years). Controls included two patients who received highly cross-linked, gamma-inert sterilized polyethylene components with neither radiographic osteolysis nor intraoperative osteolysis (2.9–5.2 years, 4.1 years).

Radiograph Scores

To determine the extent and location of osteolysis, serial anteroposterior and lateral radiographs of the affected hip joint were scored by a qualified orthopaedic surgeon. Loosened components were defined as those that demonstrated a complete lucent line on any radiograph, femoral subsidence of >2 mm, or acetabular component migration or tilt, and osteolysis was defined by lucent areas adjacent to the implanted device in either the acetabular or femoral zone. The femur was divided into seven zones and the acetabulum into three zones to evaluate the location of lucent lines of osteolysis [33].

Histomorphology and Wear Debris Imaging and Analysis

Retrieved tissues were fixed in Universal Tissue Fixative (Sakura Finetek USA, Inc.) and transferred to 70% ethanol 4 days after surgery. Representative regions were selected from each tissue, embedded in paraffin, and 6-μm serial sections were mounted onto Fisher Superfrost/Plus slides and used for histological and immunohistochemical staining. Slides were dewaxed, rehydrated, and stained with hematoxylin and eosin (H&E) (ThermoFisher Scientific). Brightfield microscopic images were visually scored for the presence of inflammation (histiocytes and giant cells) [34]. Slides were imaged using an Olympus BX50 microscope (Olympus, Melville, NY), equipped with a stepper motor controlled stage, an elliptically polarized light imaging system, and a PixeLINK camera. A representative 9-image montage was created from each tissue section in brightfield and polarized light.

Polyethylene particle number, size, and shape were determined using a customized macro in NIH ImageJ on the polarized light images based on previous studies [34–36]. In brief, polarized light images were split into three 8-bit channels (red, green, and blue). Signals from the green and blue channels were summated, and the images were converted into masks based on a threshold value relative to the average signal intensity of each image. All images were visually reviewed to ensure that false positive signals from birefringent collagen did not contribute to particle analysis results. The resulting particle number was then converted to number per mm^2 area of tissue using a measured conversion factor of 0.29 μm/pixel.

Immunohistochemistry, Imaging and Analysis

Immunohistochemistry was performed to evaluate the presence of osteolysis (HMGB1, Rabbit IgG, Abcam), reactive oxygen species (COX2, Mouse IgG1, Abcam), reactive nitrogen species (iNOS, Mouse IgG1, R&D Systems), and their oxidized products (4-HNE, Mouse IgG1, Percipio Biosciences; and NT, Mouse IgG3, R&D Systems). Optimal conditions for each antibody were determined using tissues retrieved from patients with arthrofibrosis. The antibody concentrations were: 4-HNE 1:50, COX2 1:100, HMGB1 1:200, iNOS 1:50, and NT 1:100. Before incubation at 4 °C overnight with primary antibody, the slides were incubated in an antigen retrieval solution (Vector Labs), 0.5% Triton in PBS to enhance permeability, 3% H_2O_2 in methanol to block endogenous peroxidases, and finally to block non-specific background