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Mechanical instability and titanium particles induce similar transcriptomic changes in a rat model for periprosthetic osteolysis and aseptic loosening

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ABSTRACT

Wear debris particles released from prosthetic bearing surfaces and mechanical instability of implants are two main causes of periprosthetic osteolysis. While particle-induced loosening has been studied extensively, mechanisms through which mechanical factors lead to implant loosening have been less investigated. This study compares the transcriptional profiles associated with osteolysis in a rat model for aseptic loosening, induced by either mechanical instability or titanium particles. Rats were exposed to mechanical instability or titanium particles. After 15 min, 3, 48 or 120 h from start of the stimulation, gene expression changes in periprosthetic bone tissue was determined by microarray analysis. Microarray data were analyzed by PANTHER Gene List Analysis tool and Ingenuity Pathway Analysis (IPA). Both types of osteolytic stimulation led to gene regulation in comparison to unstimulated controls after 3, 48 or 120 h. However, when mechanical instability was compared to titanium particles, no gene showed a statistically significant difference (fold change $\geq \pm 1.5$ and adjusted p-value ≤ 0.05) at any time point. There was a remarkable similarity in numbers and functional classification of regulated genes. Pathway analysis showed several inflammatory pathways activated by both stimuli, including Acute Phase Response signaling, IL-6 signaling and Oncostatin M signaling. Quantitative PCR confirmed the changes in expression of key genes involved in osteolysis observed by global transcriptomics. Inflammatory mediators including interleukin (IL)-6, IL-1β, chemokine (C-C motif) ligand (CCL)2, prostaglandin-endoperoxide synthase (Ptgs)2 and leukemia inhibitory factor (LIF) showed strong upregulation, as assessed by both microarray and qPCR. By investigating genome-wide expression changes we show that, despite the different nature of mechanical implant instability and titanium particles, osteolysis seems to be induced through similar biological and signaling pathways in this rat model for aseptic loosening. Pathways associated to the innate inflammatory response appear to be a major driver for osteolysis. Our findings implicate early restriction of inflammation to be critical to prevent or mitigate osteolysis and aseptic loosening of orthopedic implants.

1. Introduction

Despite a high success rate for hip and knee arthroplasty, the number of patients in need of revision is estimated to increase as a result of long-term implant failure (Kurtz et al., 2007) primarily due to aseptic loosening (Ulrich et al., 2008; Aujla and Esler, 2017). Bisphosphonates and TNF (Tumor necrosis factor)-blockers have been tested to alleviate ongoing bone resorption at the bone-implant interface and the consequent implant loosening, but failed to prove effective. Only when given intraoperatively, bisphosphonates seem to prevent resorption and early loosening (Schilcher et al., 2017). For established loosening, surgical intervention is thus the only option to restore function after aseptic loosening of orthopedic implants.

In the tissue surrounding loosened prostheses, the osteoclast-osteoblast balance is disrupted in favor of bone-resorbing osteoclasts. Higher ratios of osteoclast/osteoblast numbers have been observed in bone tissue from patients with osteolysis around loosened compared to well-fixed prostheses (Kadoya et al., 1996). In the tissue surrounding loosened implants, TNF (Xu et al., 1996), IL (Interleukin)-1β (Kim et al., 1993), IL-6 (Sabokbar and Rushton, 1995), CCL (Chemokine (C-C motif) ligand) 2 and CCL3 (Nakashima et al., 1999) and other mediators are present. Macrophage activating marker CHIT-1 (Chitinase 1), and IL-8, as well as osteoclast differentiation and function markers like DC-STAMP (Dendritic cell-specific transmembrane protein), TRAP (Tartrate-resistant acid phosphatase) and Cathepsin K were found elevated in periprosthetic tissue of loosened implants (Koulouvaris et al., 2008).

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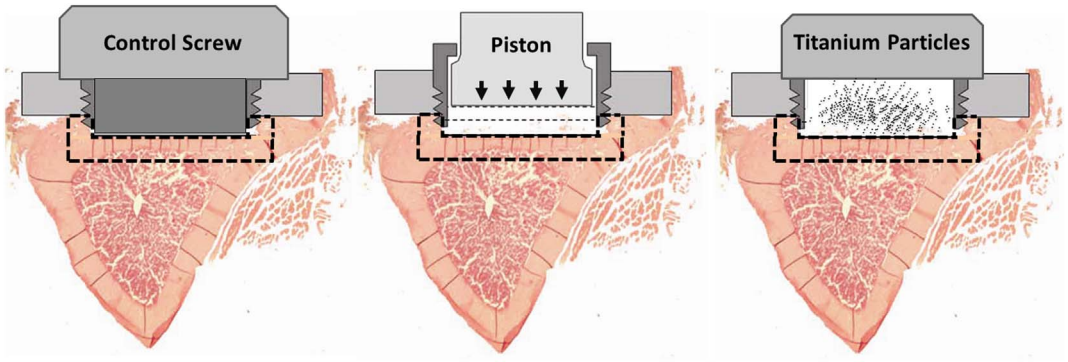

Fig. 1. Validated rat model for aseptic loosening. The animal model allows for application of particles, representing wear debris particles, or simulation of mechanical instability corresponding to implant micromotion. The marked area indicates where samples were harvested for RNA isolation.

In aseptically failed total knee arthroplasty (TKA) and total hip arthroplasty (THA), differences in CCL3, NFκB (Nuclear factor kappa-B ligand) and DC-STAMP levels were found between TKA and THA, suggesting that different mechanisms might underlie bone degradation and aseptic loosening depending on location of implant (Tomankova et al., 2014).

Bone resorption induced by wear debris particles is well characterized. A foreign body response, mediated primarily through phagocytic activity of macrophages, plays a key role along with a plethora of inflammatory cytokines and chemokines contributing to the immune reaction and recruitment of inflammatory cells to the site (Ingham and Fisher, 2005; Veronesi et al., 2017). Inflammatory mediators elevate RANKL levels which is a crucial osteoclastogenesis inducer (Ingham and Fisher, 2005). In macrophage cultures, exposure to particles induces several inflammatory mediators, known to promote osteoclast differentiation (Horowitz and Gonzales, 1997; Garrigues et al., 2005). The number and function of osteoblasts is also reported to be negatively affected by different types of particles (Lochner et al., 2011; Queally et al., 2009; Atkins et al., 2009). Moreover, human mesenchymal stem cells have shown lower survival rate (Wang et al., 2002) and blunted osteogenic differentiation capacity (Okafor et al., 2006) after exposure to titanium particles.

Apart from wear debris from prosthetic surfaces, mechanical factors like instability of implants leading to micromotion may induce fluid pressure changes resulting in fluid flow, which has been associated with aseptic loosening (Gallo et al., 2013; Sundfeldt et al., 2006). Clinical case studies have linked fluid flow (Anthony et al., 1990) and fluid pressure (Walter et al., 2004; Robertsson et al., 1997) to osteolytic lesions around orthopedic prostheses. Substantial increases in RANKL/OPG (Osteoprotegerin) ratio mRNA levels have been reported following ex vivo loading of human bone cores mimicking the mechanical condition at bone-implant interfaces (Stadelmann et al., 2008). Studies in animal models for aseptic loosening have highlighted the role of implant micromotion and fluctuating fluid flow in periprosthetic bone resorption (Aspenberg and Herbertsson, 1996; van der Vis et al., 1998; Van der Vis et al., 1999; Skripitz and Aspenberg, 2000; Jones et al., 2001; Skoglund and Aspenberg, 2003; Fahlgren et al., 2010). We previously (Nilsson et al., 2012) reported that, in a clinically relevant rat model for aseptic loosening (Skripitz and Aspenberg, 2000; Fahlgren et al., 2010), titanium particles and mechanical instability of implants induced a similar extent of osteoclast differentiation assessed by immunohistochemistry and osteoclast numbers. Although periprosthetic osteolysis induced by particles has been investigated extensively, biological mechanisms through which mechanical instability leads to osteolysis are not fully clarified. Here, for the first time, we compared the in vivo gene expression patterns following exposure to either mechanical instability or titanium particles through global transcriptome profiling in our validated animal model for aseptic loosening.

2. Materials and methods

2.1. Animal model

This study was approved by Linköping animal experiments ethical committee (ethical # 85-12) and all experiments were carried out in accordance to guidelines for care and treatment of experimental animals recommended by the committee. In total, 66 male 11-week-old Sprague-Dawley rats, weighing approximately 370 g (SD = 17) at the start of the experiments, were used. Two rats were housed in each ventilated cage in 12-hour light/dark cycle with access to food and water ad libitum. A validated animal model for aseptic loosening, described in detail previously (Skripitz and Aspenberg, 2000; Fahlgren et al., 2010), was used to either induce mechanical instability or administer titanium particles.

Briefly, following general anesthesia induced by 5% isoflurane and preoperative subcutaneous injection of 20 mg/kg Engemycin and 7 mg/kg Carprofen, the top surface of the cortical bone on the right proximal tibia was milled down and a titanium plate was fixed on the bone and left to osseointegrate. The plate had a central plug that could be removed to allow access to the bone surface. After 5 weeks of osseointegration a second surgery, with preoperative subcutaneous injections of 20 mg/kg Engemycin and 0.04 mg/kg Temgesic was performed. And the plug was removed and replaced with either an instability piston to induce mechanical instability corresponding to implant micromotion, or a hollow screw containing titanium particles to simulate wear debris particles released from prosthetic surfaces (Fig. 1).

With the instability piston in place, there was a 1-mm space between the piston and the bone. By pressing on the piston manually through the skin, it could be moved 0.5 mm down to pressurize the fluid in the 1-mm space. This creates a flow propagating through and along the underlying bone. The instability piston does not reach the bone surface during instability episodes (a 0.5-mm gap remains), avoiding risk of microfractures. To induce mechanical instability, 20 displacement cycles with a force of 8 N at 0.17 Hz, during 2 min (Fahlgren et al., 2010), were applied twice a day under anesthesia. Control animals underwent the same surgery and the central plug was removed from their implants before sample collection but they were not administered particles or implant displacement.

The moving parts of the piston are isolated from the bone by a silicone membrane, and previous histological evaluations of bone tissue under the implant have shown that induction of mechanical instability does not release any debris particles from implant surfaces (Skripitz and Aspenberg, 2000; Fahlgren et al., 2010; Nilsson et al., 2012). When harvesting, samples from groups exposed to titanium particles were evaluated macroscopically, confirming that particles were spread over the bone surface. All animals were in good health throughout the experiments period and no signs of infection were observed at any time.