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The key role of proinflammatory cytokines, matrix proteins, RANKL/OPG and Wnt/β-catenin in bone healing of hip arthroplasty patients

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ABSTRACT

Introduction: We still lack understanding of why some implants fail while most remain stable after decades of use. Proinflammatory cytokines, matrix proteins and bone regulating cytokines of the RANKL/OPG (receptor activator of nuclear factor kappa B ligand/osteoprotegerin) and Wnt/β-catenin pathways are mandatory for normal bone repair but their spatial and temporal role in the healing of primary total hip arthroplasties (THA) has not been previously shown.

Materials and methods: Twenty-four osteoarthritis patients with one-sided well-fixed primary THA were prospectively monitored during 18 years (18 Y) with repeated blood samples, clinical variables and radiographs. Eighty-one healthy donors divided in three age- and gender-matched groups and twenty osteoarthritis patients awaiting THA and serving as control of the validity of stored plasma in THA patients, were included. Plasma was analyzed for C-reactive protein (CRP), interleukin (IL)-6, IL-8, IL-1β, tumor necrosis factor (TNF)-α, osteopontin (OPN), secreted protein acidic and rich in cysteine (SPARC/osteonectin), osteocalcin (OC), bone specific alkaline phosphatase (BALP), N-terminal propeptide of collagen type I (P1NP), RANKL, OPG, the Wnt agonistic ligands (Wnt)-1 and Wnt-3a, and the Wnt antagonists sclerostin, Dickkopf (Dkk)-1, Dkk-3, Dkk-4, secreted frizzled related protein (sFRP)-1, sFRP-3 and Wnt inhibitory factor-1 (Wif-1).

Results: Inflammatory mediators in arthroplasty patients (CRP, IL-6, OPN) increased significantly on day one after surgery vs preoperative value (PR) and healthy subjects and returned to baseline at 6 W. TNF-α did not change relative preoperative level or healthy subjects. SPARC and OC increased in a biphasic fashion with the primary phase beginning shortly after surgery and lasting 3 M (SPARC) and 2 Y (OC) while the secondary phase peaked at 1 Y (SPARC) and 13 Y (OC), with both returning to basal level at 15 Y. BALP peaked at 3 M after surgery with a return to basal level at 2 Y followed by a continuous increase from 5 Y until 18 Y. P1NP increased immediately after surgery and returned to basal level at 6 W followed by a new peak at 10 Y returning to basal at 13 Y. IL-8 and IL-1β peaked at 5 Y post-THA and returned to basal level at 10 Y. RANKL/OPG and Wnt/β-catenin remained at preoperative levels until 5 Y post-THA when a sustained increase in OPG level, paralleled by a sustained decrease in sclerostin, started and lasted until 18 Y. Despite a strong increase by RANKL at 13 Y, the OPG/RANKL-ratio remained high between 5 Y and 18 Y. Dkk-1 and sFRP-1 remained at basal level until 5 Y followed by a peak at 7 Y and a return to basal level at 15 Y. Similarly, RANKL increased after 5 Y, peaked at 13 Y and returned to basal levels at 18 Y, thus coinciding with Wnt-1. In contrast, Wnt3a, Dkk-3, Dkk-4, sFRP-3 and Wif-1 did not differ from preoperative levels or healthy subjects during the course of the follow-up.

Conclusion: The primary peak of proinflammatory cytokines involved in the initiation of bone healing after trauma is in line with previous results. The primary phase of increased matrix proteins, P1NP and BALP paralleled by RANKL, OPG and Wnt/β-catenin remaining at preoperative level until 5 Y, support a strong formation of mineralized matrix and to a lesser degree bone during this phase. The secondary proinflammatory peak at 5 Y is likely a trigger of coupled bone remodeling and neosynthesis as it is followed by increased levels of the bone anabolic turnover marker, BALP, and mediators of the RANKL/OPG and Wnt/β-catenin pathways. A continuous increase by OPG level and the bone turnover marker, BALP, lasting from 5 Y until 18 Y and paralleled by a similar decrease in sclerostin level support their being key regulators of bone anabolism, whereas the transient and opposed activities of RANKL, Wnt-1, Dkk-1 and sFRP-1 serve as fine tuning tools during the coupled remodeling phase.

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1. Introduction

Despite a considerable number of in vitro studies investigating the immune responses to wear debris, we are still in the dark as to why wear generated in the vicinity of all hip implants only causes a limited number of implants to fail whereas a majority survive 20 years of constant use without revision [1]. A key to understanding the molecular mechanisms triggering prosthesis loosening is the understanding of the biological processes enabling implants to stay stable. In the current study in patients with stable hip implants, we focused on the role of proinflammatory cytokines, bone matrix and mineralization proteins, bone turnover markers and two major bone formation and remodeling pathways, RANKL-OPG [2] and Wnt/β-catenin [3], all being critical to the regulation of the skeleton both in health and disease.

Many studies of prosthesis loosening have shown wear particles to be engulfed by phagocytic cells causing them to secrete proinflammatory cytokines, chemokines and proteases which are believed to play an important role in periprosthetic osteolysis by aiding in the differentiation, maturation and activation of osteoclasts [4]. The role of proinflammatory cytokines is however multifaceted as they may both play a primary role in triggering the onset of the destructive processes characteristic of osteolysis but are also critical for the initiation of bone repair [5]. An early phase of bone healing is the formation of a meshwork of non-collagenous proteins in the area of the damaged bone, osteoid, which functions as a scaffold for bone cells. This soft callus is gradually mineralized to form the hard callus which undergoes remodeling through the actions of osteoblasts, osteocytes and osteoclasts before being replaced by trabecular and lamellar bone [5]. A pathway playing an important role in bone remodeling and neosynthesis is the RANKL-OPG axis which attracted much interest in connection with prosthesis loosening [2]. RANKL is produced by osteoblasts and binds to the RANK receptor on the surface of preosteoclasts triggering their transformation into mature osteoclasts and inducing their activation. OPG, the natural circulating antagonist of RANKL, is produced by various cell types in addition to chondrocytes and osteoblasts [2] and acts as a counter-balance to RANKL-induced osteoclastic activity. Imbalance in the RANKL-OPG axis has been considered a major cause for osteolysis and aseptic prosthesis loosening [6] and, although simple in appearance, the pathway has a complex cross-talk with other signaling cascades and is under regulation by numerous immune factors [2]. In contrast, studies addressing the importance of Wnt signaling in prosthesis loosening have only recently begun to appear and show that particle-induced osteolysis involve suppression of Wnt mediated osteogenesis [7], whereas activation of canonical Wnt is able to suppress wear induced osteolysis [8,9]. Wnt signaling has traditionally been divided in three subgroups, where the canonical Wnt/β-catenin pathway has emerged as the predominant actor in skeletal regulation. This pathway is activated by a number of circulating agonistic ligands (e.g. Wnt-1, Wnt-3a) which induce an intracellular downstream cascade causing cytoplasmic β-catenin to stabilize, as it otherwise undergoes enzymatic degradation, and allows it to translocate to the nucleus where it activates transcription genes [3]. A number of intracellular and surface-active secreted antagonists counter-balance the activity of the ligands and act either by binding directly to ligands and blocking their ability to connect with their receptor or by competing with ligands for their receptor site [3]. Wnt/β-catenin, which affects all three types of bone cells has both direct bone anabolic effects by enhancing the commitment of mesenchymal stem cells into osteoblasts and osteocytes and indirect by stimulating their production of OPG which suppresses the activity of osteoclasts [3].

The tight interdependence and interplay between various systems and pathways in bone repair has been the focus of many studies [2,5,10] but, despite having direct clinical relevance for arthroplasty patients, their joint role in bridging and sustaining bone tissue around stable implants has to the best of our knowledge not been previously described. Our aim was to monitor the bone healing process of clinically and radiologically stable primary hip implants by repeated measurements of plasma biomarkers over a period of two decades and to relate these changes to pre-surgery levels and to gender/age-matched healthy individuals.

2. Materials and methods

2.1. Study design and study population

The study was approved by the institutional review board and informed consent was obtained from every patient prior to inclusion. This study, with level of evidence II, complies with the STROBE-statement for observational studies [11]. (I) Sixty consecutive patients scheduled for primary THA due to osteoarthritis (OA) were enrolled into the study to evaluate different femoral stem designs with clinical parameters, radiography, radiostereometry and dual-energy x-absorptiometry [12,13]. In addition, venous blood was sampled on each visit. The THA group received either of two uncemented stems i.e. Epoch®, a low-modulus stem with porous coating and reduced stiffness ($n = 10$) and Anatomic®, a titanium alloy stem with porous coating ($n = 14$), both Zimmer-Biomet, Warsaw, Indiana, USA. Both stem types were supplied with an additional layer of hydroxyapatite and tricalciumphosphate (HA/TCP) on the porous coating. The Anatomic stems were also coated with pure hydroxyapatite distal to the porous coating [16]. All patients received an uncemented porous press-fit cup with HA/TCP coating (Trilogy®, Zimmer-Biomet) on the acetabular side fixed with or without additional screws. Characteristics, such as wear rates, stem migration pattern, loss of BMD and clinical outcome, were reported for the two stem implants in a 7-year follow-up [12]. Basic demographic data, comorbidities and medications were registered. Fourteen patients with a hip implant inserted on the contralateral side before the index operation and twelve patients who received a contralateral total hip implant during the course of the follow-up, were excluded. Eight patients with lysis or implant wear prompting revision surgery and two patients with clear-cut lysis, but no revision, were excluded, leaving a total of 24 patients with radiographically well-fixed implants on one side (mean age 58 Y, range 40–69; 16 males and 8 females). (II) Twenty OA patients awaiting THA (mean age 58 Y, range 34–86; 14 males and 6 females), were recruited to control the validity of biomarkers in stored plasma. (III) Eighty-one healthy subjects served as controls to THA patients over time and were divided into three subgroups (Tables 1 and 3). The groups of healthy were chosen to represent an entry, a midpoint and an exit point in the follow-up of THA patients and were matched for age and sex. We did not investigate socioeconomic status. Exclusion criteria in both the THA group, the control group awaiting THA and the healthy groups were selected to minimize risk of interference with the validity of biomarker results. We excluded subjects in all groups suffering from any kind of malignancy, immune disorders (e.g. HIV) or immune-related diseases (e.g. rheumatoid arthritis), diseases affecting the bone (e.g. Paget's disease), kidneys or liver and individuals on medication with steroids, immunotherapy or bone-regulating drugs (e.g. bisphosphonates, denosumab, calcitonin, PTH). In addition, healthy subjects were excluded if they had a history of bone trauma within a year of inclusion or diseases affecting the joints (e.g. osteoarthritis). Harris hip and pain scores were recorded during the entire follow-up. Standard plain radiography in THA patients was performed with hip and pelvis in frontal projection as well as a cross table lateral projection. Radiographs were taken before surgery, 6 weeks (6 W), 3 months (3 M), 6 M, 1 Y, 2 Y, 5 Y, 7 Y, 10 Y, 13 Y, 15 Y and 18 Y after the index operation.

2.2. Blood sampling and analysis of plasma cytokines

Venous blood was drawn into EDTA tubes from THA patients on the day before surgery (PR), one day after surgery (PO), 6 W, 3 M, 6 M, 1 Y, 2 Y, 5 Y, 7 Y, 10 Y, 13 Y, 15 Y and 18 Y post-surgery. In the three healthy