学位論文内容の要旨

博士の専攻分野の名称　博士（医　学）　氏 名　照川 ヘンド (Alhasan Hend)

学 位 論 文 題 名

Study on the regulatory function of Annexin A1 (AnxA1) in pathological bone resorption and its therapeutic implications in periprosthetic osteolysis

アネキシン A1 (AnxA1)の病的骨吸収制御メカニズム及び人工関節周囲骨吸収における治療効果に関する研究

Background and purpose: Total joint arthroplasty (TJA) is the most prosperous approach that reduces pain and restores the function of joint in patients worldwide. Periprosthetic osteolysis associated pathological bone resorption is the most common cause of arthroplasty failure. Wear debris derived from implant during the motion of the joint triggers local inflammatory response followed by pathological bone resorption resulting in mechanical instability of prosthetic components. Therapeutic targets, including bisphosphonates and monoclonal antibodies to receptor activator of nuclear factor kappa-B ligand (RANKL), tumor necrosis factor-alpha (TNF-α) or interleukin-1 (IL-1) that reduce osteoclastic-bone resorbing activity or block inflammation have failed to prevent pathological bone loss or to prolong the lifespan of implant. A better understanding of molecular and cellular mechanisms is necessary for development of novel therapeutic intervention. Macrophages play a vital role in the pathogenesis of osteolysis, as they are the predominant cells at the site of periprosthetic tissues, major source of inflammatory cytokines and they can be differentiated into osteoclasts. My earlier study showed that neutrophils play regulatory role in periprosthetic osteolysis, suppressing inflammation and pathological bone resorption triggered by polyethylene wear debris of orthopedic implants. Strikingly, annexin A1 (AnxA1), which is known as pro-resolving molecule, was found to be involved in the regulatory mechanism of neutrophils in periprosthetic osteolysis. Thus, the objective of the current research is to explore molecular function of AnxA1 in periprosthetic osteolysis as a step towards development therapeutic intervention.

Materials and Methods: Freshly isolated human neutrophils were cultured with particulate debris of implant bearing materials of conventional cross-linked ultra-high molecular weight polyethylene debris (UHMWPE) for 2 h and then harvested for cytokine quantification and gene expression analysis and Western blotting. Murine debris induced-calvarial osteolysis model was used for in vivo evaluation. AnxA1-deficient mice and function blocking antibody were used to explore the function of AnxA1 in inflammatory osteolysis. For treatment experiment, following implantation of UHMWPE debris (6 mg), mice were injected onto their calvarial bone with 5 injections of N-terminal AnxA1 (Ac2-26), or 3 injections of FPR2 antagonist (WRW4). A group of mice were treated by Ac2-26 after implantation of UHMWPE debris and interperitoneally injected (5 times in parallel to Ac2-26) with PPAR-γ antagonist (GW9662). Calvariae were collected on day 7 for evaluation of lesions in bone tissues. On the other hand, consecutive injections of RANKL (non-inflammatory osteolysis model) or TNF-α (inflammatory osteolysis model) for 4 days at concentration of 100 μg/kg were performed onto calvarial bone to induce calvarial osteolysis models. Changes in calvariae were analyzed by high-resolution micro-computed tomography assessment (μCT) and histopathology. For histology and histomorphometry, 10% formalin-fixed calvariae were decalcified in 10% EDTA for 1 week and embedded in paraffin. Five-micron sections were stained with hemotoxylin and eosin (HE), and tartrate resistance acid phosphatase (TRAP) staining. An injectable thermosensitive hydrogel containing AnxA1 A2-26 peptide was prepared for treatment of pathological bone resorption associated with inflammation. Image J software was used for quantitative analyses. Significant difference between the groups was determined by one-way ANOVA, followed by Tukey's multiple-comparison procedure. Human monocytes were cultured growth medium supplemented with recombinant macrophage colony-stimulating factor (MCSF) plus RANKL with or without recombinant AnxA1 for osteoclast and bone resorption assays. Next, the transcriptional profiling of stimulated cells for 8 days was analyzed by RNA sequencing.

Results: To gain an insight into the molecular response of neutrophils to wear debris, gene expression of human neutrophils stimulated by UHMWPE debris for 2 h were analyzed. Notably, stimulated neutrophils exhibited significant upregulation of AnxA1 but not IL-10 or TGF-β. Consistently, AnxA1 was detected in synovial fluids collected from three patients undergoing revision surgery due to aseptic loosening. It is worth mentioning that the greater osteolytic lesions in neutrophil-depleted mice were associated with significant reduction in gene expression of AnxA1 in calvarial bone. In AnxA1-defecient mice, the osteolytic lesions were more severe than these in the wild type mice, suggesting the regulatory role of AnxA1 in pathological bone resorption associated with periprosthetic osteolysis. Furthermore, recombinant Anxa1 inhibited the differentiation of osteoclasts and reduced inflammatory responses of macrophages in vitro. RNA-seq data and bioinformatics revealed that treatment of macrophages with AnxA1 suppressed activation of NFκB signaling pathway and promoted the expression of genes involved in PPAR-γ pathway. To assess therapeutic application of AnxA1 for treating periprosthetic osteolysis, UHMWPE particles were implanted onto calvarial bone tissues followed by consecutive 5 local injections of N-terminal AnxA1 (Ac2-26) over 5 days. Notably, administration of Ac2-26 mimetic peptide onto calvariae significantly reduced bone loss, inflammatory cells infiltrate, and osteoclast-stained areas (TRAP) triggered by UHMWPE debris. In contrast, WRW4 treatment exaggerated osteolytic lesions induced by UHMWPE debris. Interestingly, the therapeutic effects of Ac2-26 were abrogated in mice received GW9662, suggesting that there is correlation between therapeutic mechanism of AnxA1 and PPAR-γ pathway activation. Together, these data demonstrated that AnxA1/PPAR-γ axis may play regulatory role in attenuating inflammation and pathological bone resorption induced by implant wear debris. To further gain a better insight into the mechanism of action of AnxA1 in pathological bone resorption, TNF-α- and RANKL-induced bone loss models were used. Remarkably, Ac2-26 peptide treatment resulted in significant reduction in bone loss, inflammatory cells infiltrate, and osteoclast-stained areas in the two osteolysis models. Likewise, a single administration of the Ac2-26-mixed Matrigel onto calvariae (beyond lambdoid suture) suppressed the osteolytic lesions and the pathological bone resorption induced by the TNF-α administrations.

Discussion: Chronic inflammation occurred at the site of implant plays crucial role is this pathological condition as it negatively affects bone metabolism and promotes bone resorption resulting in a loss of implant fixation. There is a growing body of evidence suggesting that controlling chronic inflammation at the implant site would be a promising approach for therapeutic intervention. AnxA1 is an endogenous pro-resolving mediator that is abundantly expressed in inflammatory exudates with the potential for serving as a therapy for a variety of inflammatory diseases. This study highlights AnxA1 as clinically-translatable therapeutic agent for managing diseases typified by pathological bone resorption. Macrophages treated by AnxA1 exhibited elevation in the expression of PPARγ and reduction in NFκB signaling pathway. In line with these findings, activation of PPARγ has been reported as promising approach for treatment of inflammatory diseases and cancer through reducing NFκB p65 transcriptional activity. In fact, activation of PPARγ derives polarization of monocytes to M2 macrophages with anti-inflammatory properties and inhibits RANKL- and TNF-α-mediated osteoclast differentiation. In analogous fashion, IL-4 inhibits RANKL-induced osteoclast formation though suppressing NFκB activation mediated by PPARγ. Therefore, the possible inhibitory effects of AnxA1 on inflammation and osteoclast differentiation might be due to its ability to decrease activation of NFκB signaling which is known as positive regulator of pro-inflammatory function of macrophages and bone resorbing function of osteoclasts. These results revealed that AnxA1/PPARγ axis seems to play an important role in attenuating inflammation and pathological bone resorption.

Conclusions: The present study sheds a light for the first time on the functional and cellular associations between neutrophils and pathophysiology of inflammatory osteolysis, delineating new strategies for innovative therapeutic approaches for prevention of implant failure. AnxA1/PPARγ axis seems to play an important role in attenuating inflammation and pathological bone resorption in periprosthetic osteolysis. The safety and efficacy of AnxA1 make it a novel clinically-translatable therapeutic agent for prevention of implant loosening.