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The role of calcitonin receptor signalling in polyethylene particle-induced osteolysis

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ABSTRACT

The detection of peptides from the calcitonin (CT) family in the periarticular tissue of loosened implants has raised hopes of opening new regenerative therapies in the process of aseptic loosening, which remains the major cause of early implant failure in endoprosthetic surgery. We have previously shown the roles of α-calcitonin gene-related peptide (α-CGRP) and the CALCA gene which encodes α-CGRP/CT in this process. To uncover the role of direct calcitonin receptor (CTR) mediated signalling, we studied particle-induced osteolysis (PIO) in a murine calvaria model with a global deletion of the CTR (CTR-KO) using μCT analysis and histomorphometry. As expected, CTR-KO mice revealed reduced bone volume compared to wild-type (WT) controls ($p < 0.05$). In CTR-KO mice we found significantly higher RANKL (receptor activator of NF-κB ligand) expression in the particle group than in the control group. The increase in osteoclast numbers by the particles was twice as high as the increase of osteoclasts in the WT mice (400 vs. 200%). Changes in the eroded surface and actual osteolysis due to ultrahigh-molecular-weight polyethylene particles were similar in WTs and CTR-KOs. Taken together, our findings strengthen the relevance of the OPG/RANK/RANKL system in the PIO process. CTR seems to have an effect on osteoclast differentiation in this context. As there were no obvious changes of the amount of PIO in CTR deficiency, regenerative strategies in aseptic loosening of endoprosthetic implants based on peptides arising from the CT family should rather focus on the impact of α-CGRP.

1. Introduction

Aseptic loosening mediated by ultrahigh-molecular-weight polyethylene (UHMWPE) wear particles still remains the major cause of early implant loosening in endoprosthetic surgery [1]. The detection of neuropeptides such as α-calcitonin gene-related peptide (α-CGRP) in the synovial fluid and periarticular tissue of loosened implants gives reason to presume an impact in the process of UHMWPE particle-induced osteolysis (PIO) [2,3]. Furthermore, nerve fibers containing peptides arising from the calcitonin (CT) family have been described in the vicinity of arthritic joints in patients with painful osteoarthritis [3,4]. Calcitonin is a 32-amino-acid peptide, which is generated as an analog to α-CGRP by alternative splicing of the CALCA gene [5]. CT was known as one of the first inhibitors of osteoclastic bone resorption. It acts via the calcitonin receptor (CTR), a G protein-coupled receptor found, for example, on the cell surface of osteoclasts, and was originally used in conditions such as osteoporosis [6–8].

The development of knockout mouse models with an isolated or combined deficiency of CT/α-CGRP provided a tool for investigating bone turnover and evaluating the impact of these neuropeptides [5,9,10]. In an attempt to assess the impact of CT/α-CGRP in the presence of UHMWPE particles, mice with an isolated or combined deficiency of α-CGRP and CT were studied [11–13]. While UHMWPE particles revealed pronounced osteolysis in wild-type mice, different observations in α-CGRP- and CT-deficient mice were made [14]. In the presence of UHMWPE particles, a reduced

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osteolysis was found in α-CGRP-deficient mice. This favored the theory of an α-CGRP-dependent effect on bone turnover in the process of aseptic loosening [13,15]. However, the observed reduction in PIO in α-CGRP-deficient mice could have been attributed to a compensatory effect of CT. Huebner et al. [16] reported the dual action of CT, as an inhibitor of both bone formation and bone resorption, which could be highly relevant in conditions associated with increased bone resorption, such as PIO. Therefore, further investigations on $Calca^{-/-}$ mice with a combined deficiency in α-CGRP and CT were performed and showed pronounced osteolysis in UHMWPE particle-treated mice at the age of 3 months, while additional substitution of CT in these animals revealed a reduction in PIO [11]. Thus, activation of the CTR could play a crucial role in the PIO process. Additionally, there have been reports on a reduction in CTR in the presence of an inflammatory response [17].

Therefore, the aim of the present study was to gain further insights into the impact of CTR-mediated signalling in the PIO process in an established murine calvaria model using a mouse strain with a global >94% deletion of the CTR [7,18].

2. Materials and methods

2.1. Animals

All experiments were performed and registered in accordance to the local authorities (Reference number: G 1073/09). In the present study, the previously described murine calvaria model of UHMWPE particle-induced osteolysis [12,13,19,20] was used in 10 male mice with a global CTR deletion (CTR-KO) generated using the Cre/loxP system and with a genetic background of C57BL/6, as reported by Davey et al. [7]. For comparative analysis, 10 male C57BL/6J wild-type (WT) mice, aged 3 months, obtained from Jackson Laboratories (Bar Harbor, Maine, USA), were similarly treated. Animals were divided into four groups ($n = 5$ animals per group). Groups 1 (WT-SHAM) and 3 (CTR-KO-SHAM) received sham surgery only, whereas the animals in group 2 (WT-UHMWPE) and 4 (CTR-KO-UHMWPE) were additionally treated with UHMWPE particles, as reported below. During the experimental period, food (V 1534-300, ssniff Spezialdiaeten, GmbH, Soest, Germany) and water were supplied ad libitum. The animals were kept under specific-pathogen-free conditions. All animals were sacrificed after a period of 14 days.

2.2. Particles

For particle-treated mice, pure UHMWPE particles (Ceridust VP 3610) obtained from Clariant (Gersthofen, Germany) were used [12]. The morphological characteristics of these particles have been published previously [21]. The mean particle size (given as equivalent circle diameter) was 1.74 ± 1.43 μm (range 0.05–11.06 μm) More than 34% of the particles were smaller than 1 μm, 50% were smaller than 5 μm and 90% were smaller than 9 μm. To avoid contamination with endotoxins, the particles were washed twice in 70% ethanol for 24 h and tested with a quantitative Limulus amebocyte lysate assay (Charles River, Kent, United Kingdom). The detection level of <0.25 EU ml^{-1} was accepted as negative.

2.3. Surgical procedure

All mice were anaesthetized with an intraperitoneal injection of 100 mg kg^{-1} ketamine (CEVA, Sante animale Ketaminhydrochlorid, Düsseldorf, Germany) and 10 mg kg^{-1} xylazine (CEVA). After exposing the periosteum using a 10 mm incision over the calvarian sagittal midline suture, the mice of groups 2 and 4 received approximately 30 μl (2×10^8 particles per ml) of dried UHMWPE particles implanted periostally, whereas groups 1 and 3 underwent sham surgery without particle implantation. The incision was closed identically in all groups using a 4-0 Ethilon skin suture (Ethicon, Sommerville, NJ, USA) [20]. After 14 days, the animals were sacrificed in a CO_2 chamber.

2.4. Microcomputed tomography

To evaluate microstructural differences of the different calvaria, high-resolution microcomputed tomography (μ-CT; SkyScan 1072; SkyScan, Aartselaar, Belgium) was performed as described previously [22]. Mouse skulls were fixed in formalin and placed in a tightly fitting rigid plastic tube inside the scanner's chamber on a computer-controlled precision stage. During scanning, specimens were rotated in equiangular steps of 0.9°. The resolution was set to 19 μm, with a given source voltage of 80 kV and a current of 100 μA. To obtain three-dimensional images, the program Cone-beam Reconstruction (SkyScan, Aartselaar, Belgium) was used, followed by further processing using a CT analyzer (CT An, SkyScan) to provide quantitative analysis of the PIO. A rectangular region of interest (ROI) of 2 mm × 6 mm centered on the midline suture was placed in one of the 2-D-reconstructed cross-section slices. The parameters of the osseous microarchitecture were obtained within a volume of interest (VOI), as described before [22].

Given the osseus microarchitecture of calvaria, the present study focused on analyzing the bone volume/tissue volume ratio (BV/TV) to detect any changes occurring in the UHMWPE-/sham-treated animals. Furthermore, 3-D images of the calvaria were generated by applying the programs CT An and CT Vol (SkyScan) using a rectangular VOI.

2.5. Histology and histomorphometry

After sacrifice, calvaria were removed as an elliptical plate of bone defined by the foramen magnum, auditory canals and orbits to fascilitate histological processing. To protect the calvaria and prevent artifacts, the skin was kept while the brain tissue was removed. Subsequently, the calvaria were decalcified, dissected into four cross-sections and embedded separately in paraffin blocks. The sections were cut into thin coronal slides using a Reichert-Jung microtome (Model 2065, Heidelberg, Germany) and underwent hematoxylin & eosin (HE) staining to determine the bone resorption area in the midline suture.

Thereafter, all sections were digitally photographed at a magnification of 10 × 10 with the midline suture in the center using a standard high-quality light microscope. For histomorphometric analysis of the eroded surface area, an ROI within the midline suture was encircled by the operator according to the principles of bone perimeter measurement proposed by Parfitt et al. [23] and described previously [22]. Thus, the recorded ROI was calculated automatically to quantify bone destruction based on image analysis software (UTHSCA Image Tool, IT version 3.0; University of Texas, San Antonio, TX). Bone thickness was measured at the center, at four 0.5 mm steps from the midline suture to the left and at four equivalent steps to the right (see Fig. 3 for further illustration).

The quantification of osteoclast numbers was performed on the HE-stained slides by two independent investigators in the area adjacent to and in continuity with the midline suture. Osteoclasts were identified as large multinucleated cells located within a resorption lacuna with a surrounding peripheral cytoplasm that lacks organelles, as already reported by Kukita and Kukita [24].

2.6. Serum and urine analyses

To assess calcium–phosphorus homeostasis and markers of bone turnover, urine was collected and blood samples of 250 μl

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