THE EFFECTS OF ENDOMEDULLAR IMPLANTS ON THE PERIOSTEAL VESSEL STRUCTURES OF THE RAT TIBIA

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Purpose: The healing of bones is successfully achieved after intramedullar fracture-fusing techniques after fractures or surgery despite the definite impairment of peri- and endosteal microcirculation. Prostheses used for surgical treatment of hip osteoarthritis also impair endosteal circulation. Based on these observations several authors have proposed that the connection between peri- and endosteal components of the microcirculation is of minimal importance in long canaliculated bones. Alternatively, we hypothesized that the reduced efficacy of the endosteal circulation may be overcome by a compensatory hypertrophy of the periosteal circulation.

To test this presumption, we intended to answer the following questions:
1. To what extent does the impairment of the endosteal circulation modify the density of periosteal vasculature?
2. Is there any impact of drilling the medullar cavity and placement of intramedullar implant on the density of periosteal circulation?
3. Does the implant material influence the changes in periostial vasculature?

Methods: In female Wistar rats, the medullary cavities of tibias were drilled out with a series of microdrills (after opening the proximal metaphyseal cortices of the shin bones) and implants of different material (polyethylene or titanium) were infixed tightly into the moulded cavity. Endomedullary vasculature was injured also by drilling or remained intact in the contralateral tibias. After a 3-months follow up period, the anteromedial and anterolateral surfaces of tibial periostium were exposed under an operating microscope, and the periosteal microarchitecture was examined with a Cytoscan A/R-type intravaltral videomicroscope. Vessel density of the anteromedial and anterolateral surfaces of tibial periostium was evaluated by using an image analyzer computer software.

Results: Endomedullary drilling (without implants) did not induce periosteal vascular density changes (total vessel length/examined area). Higher vascular density was observed in the anteromedial and anterolateral surfaces of tibial periostium (by 40% and 70%, respectively; p<0.001) in bones with polyethylene endomulillary implants as compared to those of the intramedullarily drilled contralateral tibias. With titanium implants, however, the vascular density in the periostium was only 20% higher (ns). A moderate, albeit not significant increase in the ratio of larger diameter vessels in the periostium was seen in response to endomedullary drilling and titanium infixing. In polyethylene implants, however, the direction of changes was opposite as the ratio of large vessels did not increase, but rather decreased by ~20%, suggesting the preponderance of smaller-diameter vessels in the periostium.

Conclusions: Surgical damage of the endosteal vessels itself did not induce significant changes in the vascular structure of the rat periostium. Placement of endomedulillar implants, however, induces an enhanced vessel formation in the periostial compartment. The quality of the implant has a strong influence on these structural changes. Specifically, polyethylene brings about a marked increase in periostial vascular density with a concomitantly increased ratio of small vessels. Our results suggest that the biologically inert endomulillary titanium causes less pronounced compensatory vessel hypertrophy in the periostium as compared to polyethylene. These results can be linked to the lower osteointegrative properties of polyethylene; such material may inhibit the regeneration of the endosteal vessels which is compensated by vascular neogenesis reactions of the periostium.

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PIOGLITAZONE, A PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA AGONIST REDUCES INFLAMMATORY-INDUCED ALTERATION OF BONE STRUCTURE IN RAT ADJUVANT POLYARTHRITIS: EVIDENCE FOR A BONE PROTECTING EFFECT IN INFLAMMATORY CONDITIONS

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Purpose: Rheumatoid arthritis is characterized by synovial hyperplasia, inflammatory infiltration, cartilage destruction and juxta-articular as well as generalized bone demineralization. Since the discovery of their ability to mediate the pleiotropic effects of xenobiatics and fatty acid peroxisome proliferators in rodents, peroxisome proliferators-activated receptors (PPARs) have emerged as key regulator of lipid metabolism in humans. PPARs are members of the nuclear hormone receptor superfamily which behave as ligand-activated transcription factors in response to endogenous fatty acids and eicosanoids or isotype-selective synthetic compounds as fibrates or thiazolidinediones. In this last decade, increased evidence has shown a role of their three isotypes in inflammatory modulation. We and others demonstrated previously that PPAR gamma agonists reduced the severity of experimental polyarthritis and the overall bone loss. In the present study, we investigated the effect of pioglitazone on inflammatory-induced demineralization and bone microarchitecture in arthritic rats, and the possible contribution of the osteoclastogenesis mediators RANKL and IL-17.

Methods: Lewis rats were sensitized by an intra-dermal injection of 1 mg of complete Freund’s adjuvant (CFA) at the basis of the tail and were treated orally for 21 days with 30 mg/kg/d pioglitazone, or with vehicle only. Arthritis severity was evaluated by clinical scoring and histological examination. Bone mineral density (BMD) of three regions of interest (lumbar spine, right and left femurs) was measured by dual-energy X-ray absorpsiometry (DEXA) before sensitization and at day 20. Micro-computed tomography (micro-CT) analysis of femur was performed to measure mean cortical height at three different points. Bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) were calculated. Circulating levels of soluble RANKL, and IL-17 were determined using commercially available immunoassays.

Results: Treatment with pioglitazone, beyond its ability to reduce arthritis severity, revealed a major protecting effect on bone erosion, as supported by the histological grading of ankle joints. Pioglitazone was effective in preventing bone resorption in arthritic animals as: i) BMD values in all ROIs of treated animals were significantly higher compared to BMD values of vehicle-treated controls, ii) femoral cortical bone thickness was preserved markedly.
in secondary spongy bone and diaphyseal cortical bone and slightly in primary spongy area, and iii) bone microarchitecture showed higher values of BV/TV, Tb.N, Tb.th and lower value of Tb.Sp in rats. Finally, we demonstrated that the overproduction of RANKL and IL-17 in sera of arthritic animals was prevented by pioglitazone treatment.

Conclusions: In the present study, we demonstrated that pioglitazone had bone protective properties in arthritis-induced inflammation. The concomitant decrease of circulating IL-17 and RANKL levels strongly suggests that pioglitazone anti-resorptive effect is likely supported by the modulation of the IL-17-RANKL osteoclastogenesis pathway.

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DEVELOPMENT OF A HUMAN OSTEOCLAST SYSTEM FOR THE ASSESSMENT OF OSTEOCLAST ACTIVITIES AND PHENOTYPES IN OSTEOARTHRITIS

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Purpose: Studies of animal models of osteoarthritis undergoing anti-resorptive therapy have suggested that inactivation of bone resorption leads to improved articular cartilage status. However, the role of osteoclasts, and subchondral bone turnover, still remains to be elucidated. In alignment, an increasing amount of research is devoted to the cellular phenotype associated with disease status. The osteoclast phenotype associated with osteoarthritis has not been investigated. We developed a pure human osteoclast system, and investigated the cellular phenotype on various bone substrates; cortical, trabecular and calcified cartilage.

Methods: CD14+ monocytes were isolated from human peripheral blood using a ficoll gradient and magnetic sorting. For investigation of osteoclastogenesis the CD14+ cells were seeded on either cortical bovine bone slices or plastic with 25ng/mL RANKL and M-CSF in the presence or absence of 17β-estradiol (0.001-10nM) and cultured for 21 days. For mature human osteoclast experiments CD14+ cells were cultured in flasks for 10 days, and the lifted and reseeded onto different matrices and cultured for another 10 days in the presence or absence of well-characterized bone resorption inhibitors (calcitonin (1nM-1μM), ibandronate (1μM) and diphyllyn (300nM)). Osteoclastogenesis was assessed using the osteoclast marker Tartrate Resistant Acid Phosphatase (TRACP), as well as TRACP staining. Bone resorption was assayed by calcium release and CTX-I (C-terminal Telopeptide of Type I Collagen) all in cell culture supernatants. Immunocytochemistry for the calcitonin receptor (CTR) and TRACP was performed on both precursors and mature osteoclasts on bone. Finally, mature osteoclasts were seeded on calcified cartilage from bovine knee joints, human trabecular bone from vertebrae and human cortical bone from femurs in the presence or absence of resorption inhibitors.

Results: Osteoclastogenesis was clearly seen when measuring TRACP, CTX-I and calcium release, with TRACP release initiated on day 5 of culture and CTX-I and calcium release on day 7. All markers were increased more than 500% (P<0.01), compared to initial values. TRACP staining confirmed the presence of large multinucleated TRACP positive cells. For the mature human osteoclasts intense bone resorption was observed within 24 hours after seeding on bovine bone slices, and bone resorption was attenuated by more than 70% in the presence of the resorption inhibitors (P<0.01). Immunocytochemistry for CTR and TRACP clearly demonstrated the presence of mature osteoclasts. Interestingly human osteoclasts also resorbed the calcified cartilage, the human trabecular and cortical bones, although differences in the level of resorption were observed, suggesting an induction of distinct osteoclast phenotypes that is dependent on the extracellular matrix at which they are activated.

Conclusions: We have demonstrated that CD14+ monocytes isolated from human peripheral blood are a highly useful source of osteoclasts, which are characterized by expression of the CTR, TRACP and bone resorption, all which are hallmarks of osteooclact function. We used this high quality osteoclast preparation to demonstrate that osteoclasts resorb calcified cartilage, and since this process is involved in the pathogenesis of OA this system can be used for characterizing osteoclast related effects on joint turnover.

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DIABETIC MICE HAD A DECREASED EXPRESSION OF DENDRITIC CELL-SPECIFIC TRANSMEMBRANE PROTEIN

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Purpose: We have reported that the impairment of osteoclast fusion had important roles for the delayed fracture healing in streptozotocin (STZ)-induced diabetic mice. In this study, we further investigated the mechanism of the malfunction of diabetic osteoclasts using genetic analysis in vivo and cell culture in vitro.

Methods: The C57BL/6 mice were irradiated (9 Gy) and injected with 4 × 106 bone marrow cells isolated from C57BL/6-EGFP mice. The diabetes was induced by intravenous administration of STZ (150 mg·kg-1·mouse). The control was injected with sodium citrate buffer. At 6 weeks after transfer, bone marrow cells were isolated from both the STZ and control groups, and then cultured in the 96-well dish with the cortical bone plate. The cortical bone plates were extraction skull bones of wild type C57BL/6, and those were fixed and dehydrated with 100% ethanol. The cell culture was performed in DMEM supplemented with 10% fetal bovine serum under 5% CO2 and 95% air at 37 °C, and the floating cell was removed after 2 h incubation. On day 3, 5, and 7, the cortical bone plates were examined to analyze the formation of the resorption pits by a scanning electron microscope. The standardized closed fracture models were created in both the control and STZ group. At 2 weeks after the fracture, the frozen sections of the fracture site were created. The osteoclasts at callus area were captured by laser capture microdissection (LCM). We extracted RNA and synthesized cDNA by using commercial kit. RT-PCR was performed to evaluate the mRNA expressions of MMP9, Cathepsin-K, Receptor activator of NF-kappaB (RANK), dendritic cell-specific transmembrane protein (DC-Stamp), and GAPDH.