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ABSTRACT OF DISSERTATION

Title	The Roles of ROS Generation in RANKL-Induced Osteoclastogenesis: Suppressive Effects of Febuxostat （RANKL が誘導する破骨細胞分化における ROS の役割と、Febuxostat による破骨細胞分化抑制効果）
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Background: Multiple myeloma (MM) has a unique propensity to almost exclusively develop in the bone marrow and generates devastating bone destruction. MM cells enhance osteoclast (OC) formation and activity and suppress osteoblastic differentiation from bone marrow stromal cells, leading to extensive bone destruction with rapid loss of bone. Receptor activator of NF-κB ligand (RANKL), a critical mediator of osteoclastogenesis, is upregulated in bone marrow stromal cells to extensively enhance osteoclastogenesis and bone resorption in MM; importantly, activated OCs in turn enhance glycolysis in MM cells and thereby MM cell proliferation, leading to the formation of a progressive vicious cycle between MM tumor expansion and osteoclastic bone destruction. Therefore, OCs should be targeted to improve treatment efficacy. Furthermore, Reactive oxygen species (ROS) is induced and plays important roles in a variety of pathological cellular processes. It's produced during RANKL-induced osteoclastogenesis from bone marrow monocyte–macrophage lineage cells (BMMs), and antioxidants, including N-acetylcysteine (NAC), have been proven to prevent the RANKL-induced OC differentiation by decreasing ROS. Anticancer agents like doxorubicin (Dox) induce excessive levels of ROS leading to cell death.

Purpose: We'd like to clarify the role of cancer-treatment-induced ROS in RANKL-mediated osteoclastogenesis and the suppressive effects of febuxostat on ROS generation and osteoclastogenesis.

Materials and methods: We used ovariectomized female mice to induce osteoporosis and compare the μ-CT results of oral administration of febuxostat with the control group. Furthermore, the analysis of osteoclast formation from murine preosteoclastic cell line RAW264.7 cells or mouse bone marrow cells, TRAP staining, bone resorption assay, quantification of ROS through the use of CellRox Green staining uder the microplate reader Spectramax i3, Reverse Transcription Polymeraze Chain Reaction (RT-PCR) and Real time-PCR, Western blot, actin ring staining and immunofluresence staining were conducted.

Results: Dox Facilitates RANKL-Mediated Osteoclastogenesis Through ROS Production and febuxostat effectively suppressed the ROS production and thereby osteoclastogenesis by Dox and RANKL in combination. Furthermore, febuxostat was able to inhibit osteoclastogenesis enhanced in cocultures of bone marrow cells with MM cells and alleviated pathological bone loss in ovariectomized mice. In addition, febuxostat rather suppressed MM cell viability without compromising Dox's anti-MM activity. Therefore, a therapeutic impact of febuxostat can be expected against cancer-induced pathological bone damage and CTIBL.

Discussion: Cancer patients have a greater chance of living longer owing to recent improvement of anticancer treatment modalities. However, bone loss emerges as one of the most serious unmet issues associated with long-term repeated treatment with anticancer agents, which can be called Cancer Treatment Induced Bone Loss (CTIB) . The present study suggests that excessive ROS production by aberrant RANKL overexpression in MM and/or anticancer treatment disadvantageously impacts bones, leading to pathological bone damage and CTIBL. ROS scavenging agents such as febuxostat may help to prevent CTIBL. The preventive activity of febuxostat against CTIBL should be validated in well-designed clinical studies.
As a dentist I believe that these findings can be of use in providing a better quality of life for patients affected by cancer as well as in the exploration of

the possibility of using them in other areas of bone loss such as those in periodontitis and peri-implantitis.