THE EFFECTS OF SRC-FAMILY KINASE INHIBITORS ON OSTEOCLASTS

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Introduction: Osteoclasts are the unique bone-resorbing cells of hematopoietic origin; their development is regulated primarily by M-CSF, RANKL and integrin-mediated interactions. Our workgroup has previously tested thousands of Src-family kinase inhibitors from the Vichem Ltd on neutrophil granulocytes, which cells show many similarities with osteoclasts regarding signaling pathways. One of the most effective inhibitors was the PD166326, an anti-leukemia drug candidate. Here we tested the effect of PD166326 on osteoclast development.
Methods: Bone marrow cells were isolated from the femurs and tibiae of C57Bl/6 mice on the 0. day and differentiated into osteoclasts in vitro in the presence of recombinant M-CSF and RANKL. Cultures were treated with PD166326 during different stages of osteoclastogenesis. Vehicle control samples received dimethyl sulfoxide (DMSO). Osteoclast differentiation was examined after 4 or 6 days by osteoclast-specific tartrate-resistant acid phosphatase (TRAP)-staining. Survival assay was performed at the 4th day using AnnexinV-PE and 7-AAD apoptosis and necrosis kit. Actin ring formation was observed with Lifeact EGFP transgenic mice.
Results: The number and the size of developing osteoclasts were strongly reduced in case of the early administration of the inhibitor. The IC50 value of the inhibitor was around 5 nM. The treatment of the cultures by 10 nM PD166326 practically blocked osteoclast development. When we administered the inhibitor later, from the 4th day – when the last main point of osteoclast development happens – only minor reduction was observed. The tendency to apoptosis did not increase in the PD166326 treated cultures. The administration of PD166326 did not cause the degradation of actin rings.
Implications: The anti-cancer drug PD166326 inhibited osteoclastogenesis in a very small concentration. We suppose that it has an effect on the early stage of osteoclastogenesis. These results may be considered in the therapy of different cancers especially in metastatic bone diseases.

I wish to hold a poster.
The subject to the abstract is of academic nature.