Targeting FAK Activation With M64HC1 Promotes Osteoclastogenesis and Bone Resorption

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INTRODUCTION: Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that orchestrates cellular processes such as adhesion, migration, and survival, and has been extensively studied in the context of epithelial repair and mesenchymal stem cell (MSC) lineage commitment. While FAK inhibition is known to impair osteoclast (OC) differentiation, the therapeutic potential of isolated FAK activation in promoting OC-mediated bone resorption remains poorly defined. In this study, we investigated the effect of M64HCl, a newly developed and selective FAK small molecule activator, on osteoclastogenesis and bone resorption using both in *vitro* and *in vivo* approaches.

METHODS: All animal studies are carried out under the approval of IACUC at NEOMED. First, to differentiate into OCs, murine osteoclast precursors (OCPs) isolated from 6–8-week-old wild-type C57BL/6J male and female mice (Strain #000664, Jackson Laboratories) were seeded on the surface of bone cortical slices and cultured with M-CSF and RANK-L in the presence or absence of various doses of M64HCl (100-500 mM). Cells were differentiated for 12-14 days before being stained with TRAP, imaged, and counted. For *in vivo* studies, Five-week-old male C57BL/6J mice were anesthetized with isoflurane and their calvariae exposed. Animals were treated with collagen sponges soaked in different concentrations of FAK activator (M64HCl) (n=4-6 per group). Animals were euthanized, and calvaria were stained for TRAP. TRAP+ OCs and the resorptive area were quantified and imaged. Animals were housed and maintained at Northeast Ohio Medical University (NEOMED) in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), under controlled conditions (21°C, 12-hour light-dark cycle). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at NEOMED.

RESULTS: FAK activation significantly increased OC differentiation, as confirmed by enhanced TRAP activity and staining, increased OC size and number, and upregulation of osteoclast-related markers including CTSK, calcitonin receptor, and OC-STAMP. Functionally, M64HCl-treated OCs exhibited greater bone resorption on dentin slices, quantified by Toluidine blue staining and ImageJ analysis (Figure 1-A). Localized delivery of M64HCl via collagen sponges in a calvaria bone resorption model led to marked increases in TRAP+ OCs and OC-mediated bone resorption (Figure 1-B).

DISCUSSION: Together, our results demonstrate that targeted activation of FAK by M64HCL potently enhances OC differentiation and function, establishing M64HCL as a promising therapeutic target for managing bone diseases characterized by pathologically increased bone mass.

SIGNIFICANCE/CLINICAL RELEVANCE: Activation of FAK by M64HCl drives the function of OC-mediated bone resorption. This may help to treat diseases characterized by accelerated bone mass, such as osteopetrosis or sclerotic conditions. Furthermore, because these molecules tip the balance to maintain active bone homeostasis, they can also be used to accelerate bone remodeling in conditions where bone remodeling is affected. Overall, this is the first study to report M64HCL small molecule FAK activator in regulating osteoclast differentiation and bone resorption. Our data suggest that M64HCL a possible therapeutic target for bone diseases with increased bone mass.

Figure-1

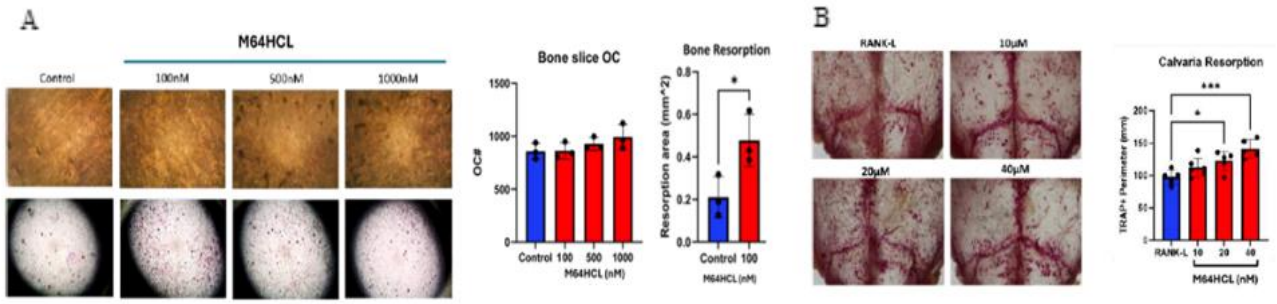

Figure 1. M64HCl promotes osteoclast activity in *vitro and in vivo*.
(A) Osteoclasts (OCs) seeded on bone slices were differentiated with or without M64HCl. TRAP staining identified mature OCs (lower panel), and Toluidine blue staining visualized resorption pits (upper panel). Resorbed area per OC was quantified.
(B) Calvariae of 5–6-week-old C57BL/6J male mice were treated with PBS (Control), RANKL (100 ng/mL), or RANKL plus M64HCl (10–40 μM) for 7 days. TRAP staining visualized OCs (left panel), and OC number and resorption area were quantified (right panel, I). Data = mean ± SEM (n = 3–6). *P < 0.05 vs. control. Scale bar = 100 μm.