

Regulation Of ITAM Adaptor And Receptor Molecules By Inhibition Of Calcineurin-NFAT Signalling During Late Stage Osteoclast Differentiation

¹Zawawi MSF, ²Dharmapatni AASSK, ²Haynes DR, ²Crotti TN

¹Department of Orthopaedics, Universiti Sains Malaysia (USM), Kubang Kerian, 16150, Malaysia

²University of Adelaide, Australia

INTRODUCTION:

Differentiation and activity of bone-resorbing cells, osteoclasts, are dependent upon receptor activator NF-kappa-B ligand (RANKL) interacting with its receptor, RANK, to induce the transcription factor, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1). The immunoreceptor tyrosine-based activation motif (ITAM)-dependent pathway has been identified as a co-stimulatory pathway in osteoclasts (1). Receptors, osteoclast-associated receptor (OSCAR) and triggering receptor expressed in myeloid cells (TREM2), pair with their adaptors, Fc receptor common gamma chain (FcR γ) and DNAX-activating protein 12 kDa (DAP12), respectively, to induce calcium signaling (2). Treatment with calcineurin-NFAT inhibitors, Tacrolimus (FK506) and 11R-VIVIT peptide (VIVIT), has been shown to reduce NFATc1 expression consistent with a reduction in osteoclast differentiation and activity (3). This study investigates the effects of inhibiting calcineurin-NFAT signalling on the expression of ITAM factors and late stage osteoclast genes including cathepsin K (CathK), Beta 3 integrin (β 3) and Annexin VII (AnnVIII).

MATERIALS & METHODS:

Human peripheral blood mononuclear cells (PBMCs) were differentiated with RANKL and macrophage colony stimulating factor (M-CSF) over 10 days in the presence or absence of FK506 at 0.01 μ M, 0.1 μ M or 0.5 μ M doses, or VIVIT at 1.0 μ M, 2.0 μ M or 5.0 μ M. Osteoclast formation was assessed at day 7 by tartrate resistant acid phosphatase (TRAP) staining and osteoclast activity was assessed at day 10 by dentine pit resorption. Gene expression was assessed at days 3, 7 and 10 by quantitative real-time polymerase chain reaction (qRT-PCR). Statistical significance ($p < 0.05$) between groups was determined by one-way ANOVA with

Tukey's Post Hoc test (dose response data analysis) and student *t*-tests (mRNA expression analysis).

RESULTS:

Osteoclast formation and activity were significantly reduced with inhibitor treatment at all doses. Gene analysis by qRT-PCR demonstrated that FK506 significantly reduced the expression of NFATc1, CathK, OSCAR, FcRc, TREM2 and DAP12 at day 10 of culture. VIVIT significantly decreased CathK, OSCAR, FcRc, and AnnVIII gene expression at day 10. No significant differences in expression of these factors were observed at earlier time points (days 3 and 7) in both FK506 and VIVIT.

DISCUSSIONS:

This data suggest inhibition of calcineurin-NFAT signalling by FK506 and VIVIT suppresses key mediators of the ITAM pathway during late stage osteoclast differentiation (day 10), and reduces both osteoclast differentiation and activity.

CONCLUSION:

Modulating ITAM related molecules may offer novel ways in inhibiting elevated osteoclast activity seen in many bone loss pathologies.

REFERENCES:

1. Hirotsani, H. *et al.*, J. Biol. Chem. 2004.
2. Alias, E. *et al.*, Acta Biomater. 2012.
3. Yu, H. *et al.*, Cardiovasc. Drug Rev. 2007.