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Pharmacological modulation of autophagy and apoptosis in human osteoclasts *in vitro*

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The aim of this study was to investigate the effect of autophagy and apoptosis modulators on osteoclast formation and function *in vitro*. Osteoclasts were differentiated *in vitro* from human peripheral blood monocytes in the presence of M-CSF and RANKL. Cells were pretreated with TNF alpha (5 ng per ml) for 24 hours before treatments. Autophagy inhibitor (Hydroxychloroquine/HCQ 50 µg/ml), autophagy inducer (Rapamycin 110 nM) and apoptotic inducer (Embelin 15 µmol/l) were administered for 24 hours. Effect of modulators was investigated using quantitative real-time PCR, immunofluorescent staining of autophagy genes (Beclin-1, LC3), apoptotic markers (caspase 3 and 9) and TUNEL. Osteoclast formation and function were investigated using tartrate resistant acid phosphatase (TRAP) and dentine resorption assay. Change in p62 level was assessed using western blot and its colocalization with LC3 was assessed using immunofluorescent staining and confocal microscope. Autophagy vesicles were visualized by transmission electron microscope and also stained with cyto ID and visualized using live cell imaging. HCQ and Embelin suppressed Beclin-1 at 6 hours and LC3 mRNA at 24 hours. Embelin induced caspase-9 at 6 hours while HCQ induced caspase-3 mRNA at 24 hours. Beclin-1 and LC3 expression, TRAP count, dentine resorption and cell size were reduced following HCQ and Embelin treatments. HCQ and Embelin treatment was associated with increased TUNEL positive cells. Live cell imaging and TEM support the evidence of apoptosis following HCQ and Embelin treatment with significant increased in accumulation of autophagy vesicles by HCQ treatment. These findings support that pharmacological modulation of autophagy and apoptosis affects osteoclast formation and function.

Biography

Kencana Dharmapatni has completed her PhD in the Discipline of Anatomy and Pathology, School of Medicine, the University of Adelaide in 2007 and MSc in Immunology, Allergy and Arthritis from Flinders University in 2002 and MBBS and MD in 1991 and 1987 in Indonesia. Currently she is a Postdoctoral Researcher and Academic in the School of Medicine and Dentistry, the University of Adelaide, Australia. She has obtained grants in arthritis and dental fields, published more than 20 papers and is a regular Reviewer for many scientific journals. She review grants for funding bodies such as NHMRC and United States-Israel Binational Science Foundation.

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