In vitro effects of the chrysin on osteoclastogenesis and resorption on RAW264.7 murine macrophages

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Introduction

Osteoclastogenesis is the formation of the bone resorbing cells called osteoclasts. Osteoclasts mature and resorb bone when stimulated by receptor activator of nuclear factor-kB (NF-kB) ligand (RANKL) binding to its receptor, receptor activator of NF-kB (RANK). RANKL leads to the activation of mitogen activated kinase (MAPK) and NF-kB signalling pathways which are crucial for the formation and function of osteoclasts. Overactive osteoclasts can lead to bone degenerative diseases such as osteoporosis.

Chrysin is a bioactive phytochemical that has been shown to possess anti-oxidant, anti-inflammatory, and anti-cancer properties. However, the potential benefits of chrysin to bone health have not been conducted.

The aim of this study was to investigate the effect of chrysin on cell viability, osteoclast differentiation, the signalling of MAPK and NF-kB pathways and the expression of key osteoclast genes (tartrate-resistant acid phosphatase (TRAP), NFATc1 and cFos) in RAW264.7 murine macrophages.

Methods

Resazurin Assay

- RAW264,7 cells were seeded at 5 000 cells/well in a 96-well plate and exposed to Chrysin (0.5, 1, 5, 10, 50, and100µM) for 24 hours. Cell viability was determined by resazurin assay. Absorbance was measured at 570 and 600 nm

TRAP Staining

- RAW264,7 cells were seeded at 5 000 cells/well in a 96-well plate and exposed to Chrysin (0.5, 1, 5, 10, 50, and100µM) and RANKL (15ng/mL). The cells are then stained and counted to determine the effect of chrysin on osteoclast differentiation. IC50 for chrysin was determined using GraphPad v7.

Western Blotting

- RAW264,7 cells were seeded at 1 000 000 cells/well in a 6-well plate and incubated for 24 hrs. They were then exposed to Chrysin (14µM) and RANKL (15ng/mL) for 5-30mins. The protein was extracted and analysed by Western Blotting to determine if chrysin had an effect on ERK signalling.

Results

Cell Viability

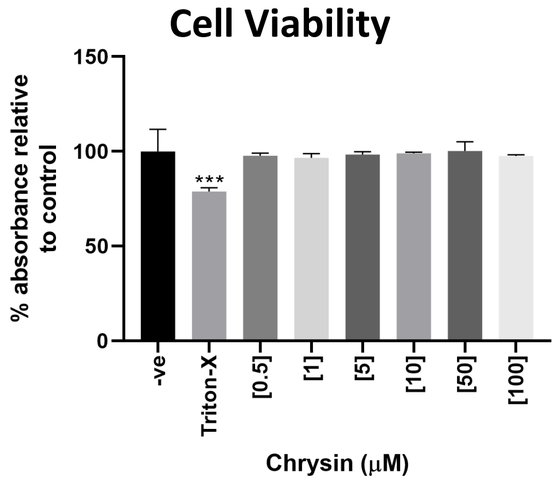

Figure 1: Chrysin has no effect on cell viability. RAW264,7 cells were seeded at 5 000 cells/well in a 96-well plate and exposed to Chrysin (0.5, 1, 5, 10, 50, and100µM) for 24 hours. Cell viability was determined by resazurin assay.
***p<0.001 vs -ve. –ve - DMSO

Osteoclast Formation

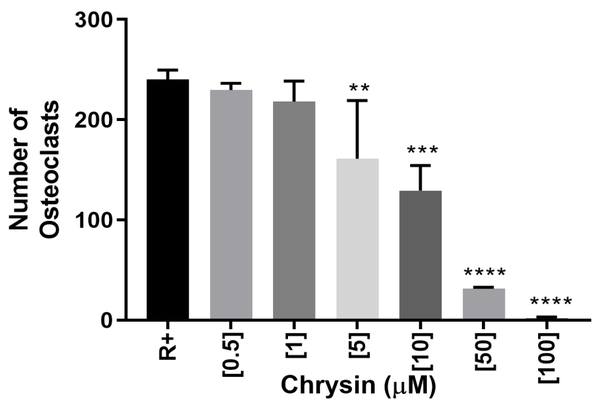

Figure 2: Chrysin reduces number of osteoclasts formed. RAW 264,7 murine macrophages were seeded at a density of 5 000 cells/well in a 96-well plate and exposed to chrysin (0.5, 1, 5, 10, 50, and 100µM) and RANKL (15 ng/mL). TRAP positive cells with 3 or more nuclei were quantified.
**p<0.01, ***p<0.001, ****p<0.0001 vs R+. R+ - RANKL

TRAP Stained Osteoclasts

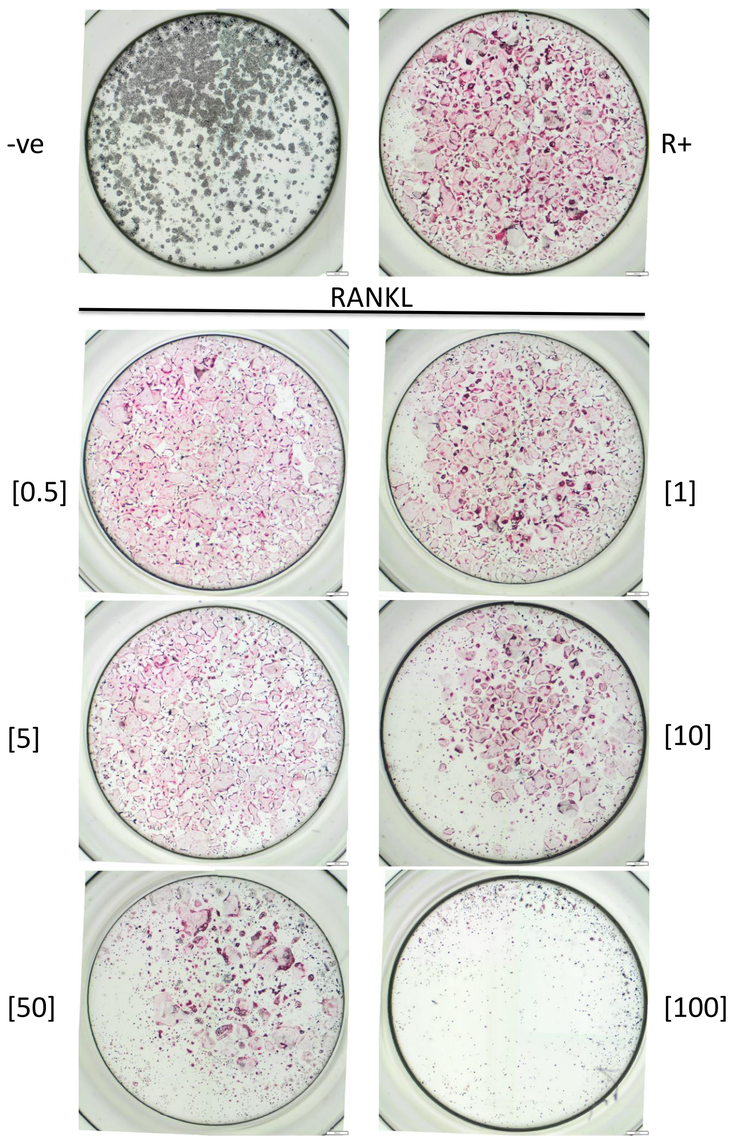

Figure 3: Chrysin reduces TRAP stained osteoclasts. TRAP positive osteoclasts with were stained and counted. Large multinucleated osteoclasts (pink stained cells) can be seen after exposure to RANKL. Fewer and smaller osteoclasts were seen after exposure to chrysin at greater than 5µM.
R+ - RANKL. –ve - DMSO

Western Blotting

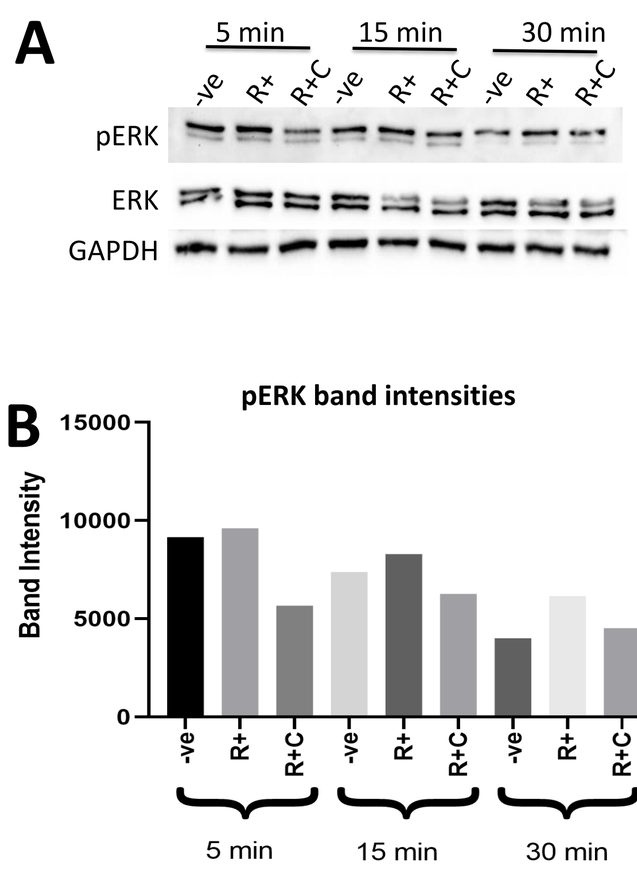

Figure 4: Chrysin targets RANKL-induced ERK signalling. RAW264,7 cells were seeded in a 6-well plate at 1 000 000 cells/well. Chrysin (14µM) inhibited the phosphorylation of ERK. **A.** Bands were visualized using a ChemiDoc MP after chemiluminescent staining. **B.** The band intensity for the pERK bands was quantified using ImageJ software.

-ve – DMSO
R+ - RANKL
R+C – RANKL and chrysin

Conclusion

- The cell viability was not significantly affected by chrysin when compared to the vehicle control in the resazurin assays.
- TRAP staining showed a significant decrease in the number of osteoclasts formed from 5 to 100µM chrysin compared to the RANKL positive treatment. The IC50 was determined to 14µM.
- Western blotting showed that chrysin (14µM) may inhibit ERK phosphorylation.
- The study suggested that chrysin can inhibit osteoclast formation possibly through inhibition of ERK phosphorylation. Future studies using qPCR will be conducted to determine the expression of key osteoclast genes.
- Chrysin may have potential as a therapeutic agent in the treatment of bone diseases characterized by overactive osteoclasts.

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