Introduction

Human osteoclasts can be generated from bone marrow-derived CD34+ mesenchymal stem cells in the presence of macrophage colony-stimulating factor (M-CSF) and ligand for receptor activator of nuclear factor kappa B (RANKL). The culture system can be conveniently used in predilutional efficacy studies for testing compounds targeted to inhibit osteoclast differentiation or activity.2

Materials and Methods

Cell culture

CD34+ human osteoclast precursor cells (PilotTech Human Osteoclast Precursors, Lonza) were cultured on bone bone slices for 7 days in the presence of M-CSF and RANKL and allowed to differentiate into bone-resorbing osteoclasts. After completion of osteoclast differentiation at day 7, the culture medium was removed and new culture medium was added into the wells. The mixture was then cultured for an additional 3 days, allowing them to resorb bone. In the osteoclast differentiation assay, denosumab (Prolia, Amgen) was added in the cultures at day 0, and the cultures were stopped at day 7. In the osteoclast activity assay, the cells were cultured without test compounds for 7 days, followed by medium change at day 7 and addition of osteoclasts (ChemSelect). The cultures were stopped at day 10.

Endpoint measurements

In the osteoclast differentiation assay, tartrate-resistant acid phosphatase 5b activity (TRACP 5b, BoneTyra) was measured in the culture medium collected at day 7 at an index of the number of formed osteoclasts.2 In the osteoclast activity assay, a general cross-linked peptides of type I collagen (CTX-I, CrossLink-i for culture ELISA, IDS Ltd) was measured in the culture medium collected at day 10 to quantify bone resorption during days 7-10.

Statistical analysis

Statistical analysis was performed with statistical software R (version 3.1.2, www.r-project.org) using one-way ANOVA. Because statistically significant differences were observed (p<0.001 between all groups), the results of all other groups were compared separately with the results of the baseline group using Tukey’s HSD test.

Results / Osteoclast differentiation

Figure 1: The effects of denosumab on differentiation of human osteoclasts in vitro. A) TRACP 5b activity (U/L) measured at day 7. COMP = Comparator group in statistics, NS = not significant, *statistically non-significant trend with p-value <0.1, **p<0.01 compared to the baseline (BL) group; B) EC50 value determination (EC50 = 0.124 µg/ml); C) and D) Representative microscopic images of the effects of denosumab on formation of TRACP positive multinuclear osteoclasts at day 7. C) Baseline (no added compounds); D) Denosumab 0.3 µg/ml, magnification x100. Denosumab blocks osteoclast differentiation at a stage where the mononuclear osteoclast precursor cells are TRACP positive, and the mononuclear osteoclast precursor cells do not secrete active TRACP 5b enzyme into the culture medium.

Results / Osteoclast activity

Figure 2: The effects of odanacatib on resorption activity of human osteoclasts in vitro. A) CTX values determined at the end of the resorption period at day 10. COMP = Comparator group in statistics, NS = not significant, ***p<0.001 compared to the baseline (BL) group; B) EC50 value determination (EC50 = 0.0433 µM); and C) and D) Representative microscopic images of the effects of odanacatib on resorption pit formation (TRITC-labeled wheat germ agglutinin lectin staining) at day 10. C) Baseline (no added compounds); D) Odanacatib 0.1 µM, magnification x100. The strong inhibition of bone resorption by odanacatib is partly due to decreasing the depth of the formed resorption pits, which is not seen in the microscopic images, indicating that CTX measurements detect more reliably total effects on resorption.

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References


Summary

Denosumab and odanacatib showed strong concentration-dependent inhibition of osteoclast differentiation and activity, respectively.

EC50 value was 0.124 µg/ml for denosumab and 0.0433 µM for odanacatib.

Denosumab and odanacatib are useful reference compounds for human osteoclast differentiation and activity, respectively.