# Potential autocrine role for serotonin in human osteoclastogenesis

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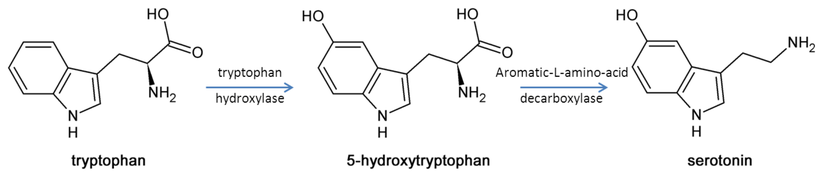
## BACKGROUND

**A role for peripheral serotonin in bone homeostasis has been proposed, yet conflicting evidence as to the mechanisms remain[1,2]. A role is evident clinically, as depression and selective serotonin reuptake inhibitors (SSRIs) a first-line treatment in mood disorders, are implicated in bone loss and fracture risk[3].**

**Transcription of genes for serotonin receptors and tryptophan hydroxylase (TPH1), a rate-limiting enzyme in serotonin biosynthesis (Fig.1), have been reported in both murine osteoblasts (OB) and osteoclasts (OC), in addition to the capacity for OC precursors to synthesise serotonin[4], however, little evidence is available in human OB and OC.**

**FIGURE 1: TPH1 IS ESSENTIAL FOR SEROTONIN SYNTHESIS**



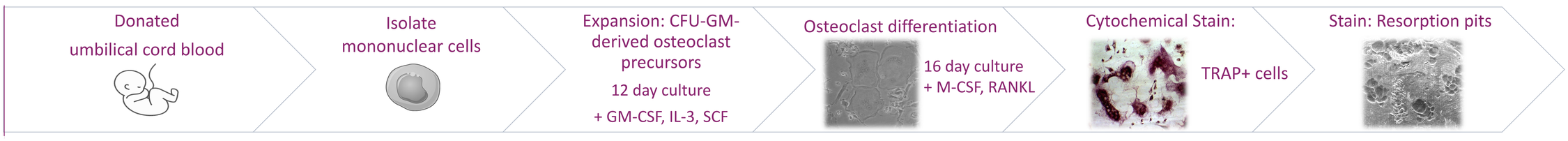

## AIM

**This study investigated the capacity of human OC to express serotonin receptor-2b (5-HTR2b) and TPH1 *in vitro* and the effects of blocking serotonin biosynthesis with a chemical inhibitor of TPH1 on human osteoclastogenesis.**

## METHODS

**Human OC were generated from umbilical cord blood (Fig.2). Colony-forming unit-granulocyte macrophage (CFU-GM)-derived OC precursors were cultured in serotonin-depleted media containing receptor activator of nuclear factor kappa-β ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) for up to 16-days and stained for tartrate-resistant acid phosphatase (TRAP). Gene transcription of 5-HTR2b, TPH1 and CATK mRNA were assessed by real time PCR. In addition, OC precursors were cultured on dentine slices in the presence of TPH1 inhibitor (LP533401-HCL) to assess OC formation and bone resorption. Data were analysed using either one-way ANOVA or two-way ANOVA, with Fisher's pairwise comparisons.**

**FIGURE 2: PROCESS OF OSTEOCLAST GENERATION AND ANALYSIS**




## RESULTS

### GENE TRANSCRIPTION IN HUMAN OSTEOCLASTOGENESIS

**TRAP-positive, multinucleated OC were observed from day-6 of culture.**

**Transcription of TPH1 was initially elevated at the commencement of the culture period (day-0). Compared to day-2, treatment with RANKL and M-CSF increased TPH1 mRNA over time, with a 3-fold increase by day-16 (p=0.016). Co-treatment with TPH1i further increased mRNA TPH1 levels with time (p=0.003; Fig.3I).**

**Transcription of 5-HTR2b also accumulated during osteoclastogenesis, with a 10-fold increase at day-16 compared to day-0 (p=<0.0001; Fig3.II). Co-treatment with TPH1i further increased mRNA levels, almost doubling that of control by day-16 (19-fold; p=<0.0001; Fig.3II).**

**Transcription of Cathepsin K (CATK) peaked at day-7 (>900-fold; p=<0.0001) and mRNA levels were maintained at day-16 (p=<0.0001; Fig3.III). Again, co-treatment with TPH1i further increased mRNA levels at each time point (1500-fold by day-16; Fig3.III).**

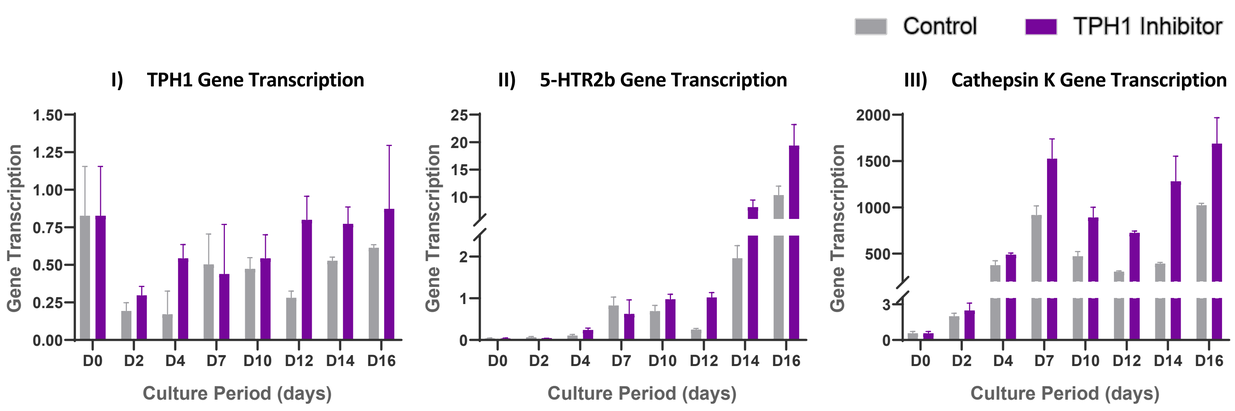



**FIGURE 3: GENE TRANSCRIPTION PROFILES IN HUMAN OSTEOCLASTOGENESIS.** CFU-GM-derived cells were cultured in serotonin-deficient media with RANKL (125ng/mL) and M-CSF (25ng/mL) in the absence (vehicle control) or presence of TPH1i at 10µM for 16 days. Levels of mRNA were quantified by real time PCR for I) TPH1, II) 5-HTR2b and III) Cathepsin K, and normalized to housekeeping gene, GAPDH. Data are presented as mean ± SEM, n=3 replicates per treatment group, gene transcription in arbitrary units.

### EFFECTS OF TPH1 INHIBITION ON HUMAN OSTEOCLASTOGENESIS

**Co-treatment with TPH1i (LP533401-HCL) dose-dependently decreased OC number, with a maximum inhibition of 59.7% at 10µM (p=0.000; Fig. 4I), whereas mean OC size was correspondingly increased (+135.4%; p=0.0001; Fig. 4II), and total resorption area per dentine slice decreased (-40.9%; p=0.003; Fig.4III) at this concentration (10µM).**

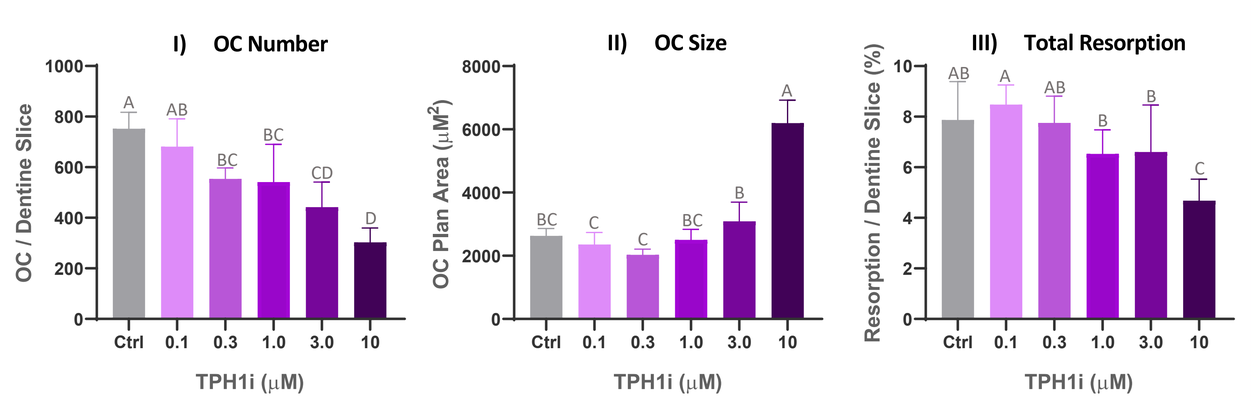



**FIGURE 4: TPH1i INCREASES OC SIZE, BUT INHIBITS RESORPTION.** CFU-GM-derived cells were cultured in RANKL (125ng/mL) and M-CSF (25ng/mL) and co-treated with various concentrations of TPH1i for 16 days. The effects on **I)** OC number, **II)** average OC size and **III)** resorption capacity were assessed. Data are presented a mean ±SEM, n=4 dentine slices/ group. Groups with different superscripts are significantly different; p<0.001; one-way ANOVA; Fisher's Multiple Comparison test.

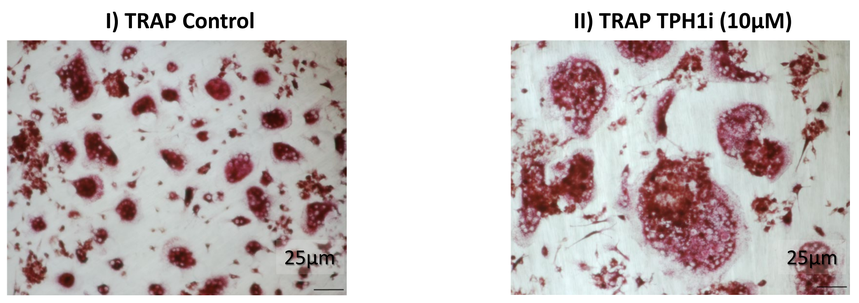



**FIGURE 5: REPRESENTATIVE PHOTOMICROGRAPHS OF THE ACTION OF TPH1i (10µM) ON OC SIZE AND RESORPTION. I)** Vehicle control, TRAP positive cells **II)** TPH1i (10µM) treated, TRAP positive cells, n=4.

## DISCUSSION & CONCLUSION

**This present human *in vitro* study supports work previously reported in animal models[4] that implicates a direct role for serotonin in osteoclastogenesis. Human OCs transcribed genes for serotonin receptor 2b and TPH1, particularly in later stages of maturation. Detection of mRNA for TPH1 indicate that human OCs may synthesize serotonin that could then signal through an autocrine pathway via cell-surface 5-HTR2b receptors. Chemical blockade of TPH1 activity increased OC size, but with a corresponding reduction in resorption capacity that occurred in an environment of increased transcription of the catabolic enzyme CATK. This seemingly counterintuitive finding may suggest that inhibition of TPH1 (and resulting decreased endogenous serotonin) may affect the process of OC fusion and attachment to bone substrate and the resulting effect on the resorptive capacity is not compensated by an increase in CATK.**

**Future work will aim to: (i) confirm protein expression and the mechanism by which inhibition of TPH1 activity influences OC fusion and resorption; and (ii) quantify secreted serotonin and the capacity of exogenous serotonin to overcome blockade by chemical inhibition of TPH1.**

**In conclusion, these findings provide evidence for a direct role for serotonin in human osteoclastogenesis and bone resorption and provide a possible mechanism via which regulators/modulators of peripheral serotonin, such as SSRIs, may impact skeletal health.**

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**ACKNOWLEDGMENTS** We acknowledge our tissue donors who generously gave their consent for our research & Deakin University for providing JRC with a Strategic Scholarship.