PTH 1-34 Effects the Migration and Differentiation of Young and Ovarectomized Bone Marrow Derived Rat Stem Cells

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Introduction

Intermittent parathyroid hormone 1-34 (teriparatide) is the N-fragment terminal of the intact hormone, currently in clinical use to treat osteoporosis. Unlike anti-catabolic agents such as bisphosphonates, PTH 1-34 not only affects the osteoclast, but also up regulates bone formation via both modelling and remodelling mechanisms. The actions of iPTH on mesenchymal stem cell differentiation (MSCs) may underpin a further method in the treatment of osteoporosis specifically, and for fracture healing in general. Stem cells from older female osteoporotic animals have reduced activity and poorer osteogenic potential; additionally, their migration to and retention at sites of increased bone turnover are reduced in comparison to cells from younger animals.

The aim of this study was to isolate bone marrow derived MSCs from both young Wild Type (WT) and ovarectomized senile (OVX) rats, then to investigate and compare the effect of pulsatile and continuous PTH administration on migration to SDF-1, proliferation and osteogenic differentiation.

Methods

MSCs were harvested from the femora of 6-9week Wistar rats, and from 10-13month ovarectomized rats with established osteopenia. Cells were cultured with 25, 50 and 100nmMol of PTH 1-34 added to osteogenic media either continuously or in a pulsatile fashion for 6 hours in every 72hour cycle. ALP and Alizarin Red were used to assess the optimal concentration of PTH for osteogenic differentiation. Subsequently, proliferation was assessed with Alamar Blue and cells were seeded in a Boyden chamber to quantify the migration to SDF-1.As the data was parametric a student t-test was used to analyse results, and a p value < 0.05 was considered significant.

Results

ALP and Alizarin Red parameters were significantly increased for both WT and OVX groups at 50nmMol of pulsatile PTH in comparison to groups cultured in 25 or 100nmMol. Continuous administration at all concentrations led to reduced calcium phosphate deposition by day 21 in all groups. Interestingly, in comparison to cells cultured in osteogenic media, 50nmMol of pulsatile PTH lead to statistically significant higher ALP and Alizarin Red measurements up to day 10 and 14 respectively in WT cells, and days 10 and 21 in OVX cells (figure 1).

The proliferation rate normalised against DNA was similar for both OVX and WT rats at all-time points. PTH administration did not effect cell proliferation in any group (figure 2.)

WT MSCs not only had improved osteogenic differentiation, but also showed increased migration to SDF-1 in comparison to OVX groups. Pulsatile PTH led to further increases in migration of both OVX and WT cells.

Conclusion

Intermittent PTH increases the osteogenic diffrentiation and migration of MSCs from both young and ovarectomised rats, though importantly this effect is not dose dependent. Ultimately, the role of PTH 1-34 on MSCs may lead to improved bone formation and cell homing capacity-particularly in the context of osteoporosis.

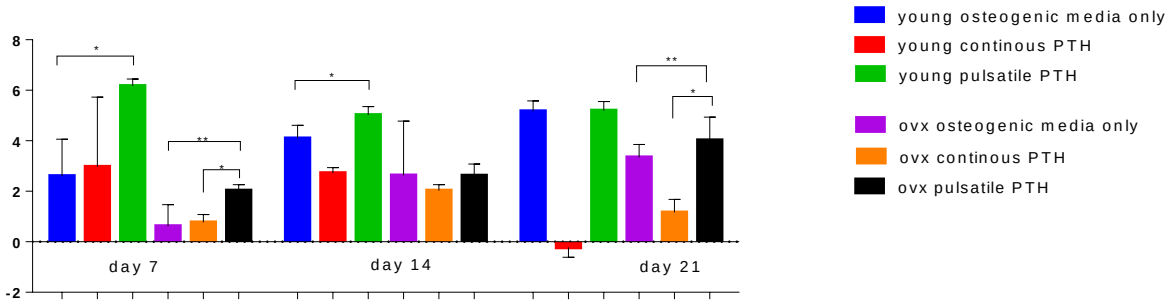

Figure 1.Alizarin Red production of young and ovarectomised cells in osteogenic media and at 50nmMol of PTH 1-34. *p=<0.001, **p=<0.05

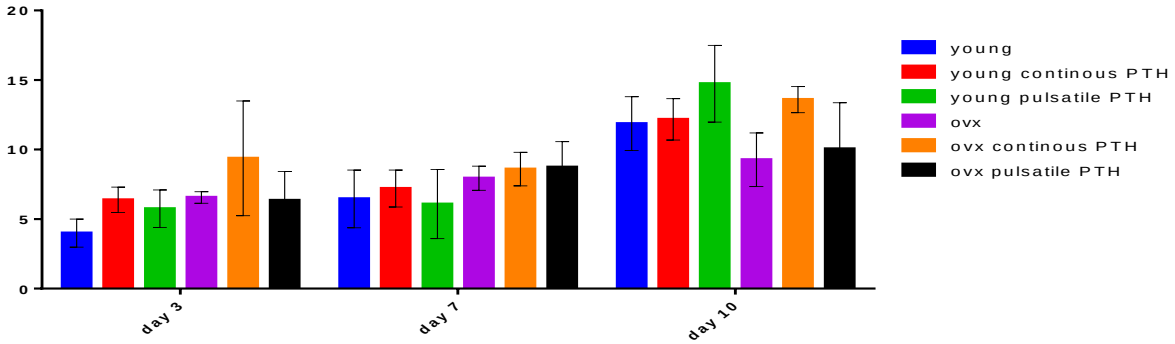

Figure 2. Alamar Blue normalised against cell DNA, showed no significant difference between WT and OVX cells, or by the addition of PTH