ASSESSMENT OF OSTEOGENIC EFFECT OF PROTOCATECHUIC ACID

Final Report: July 15, 2018
Prepared for: Lanny Johnson, MD

Objective

This is a proof of concept study to determine if Protocatechuic acid enhances osteogenesis in human osteoblasts.

Report Summary

Protocatechuic acid (PCA) increased osteogenic gene expression in a dose-dependent manner. PCA at the highest concentration tested (100µmolar) increased gene expression even greater than osteogenic media. PCA also increased expression of the growth factor BMP-6. PCA had a mixed effect on anti-osteogenic genes: reducing levels of MMP-1 and TNF-α, while increasing IL-1 and IL-6. PCA had no detectable effect on calcium deposition. PCA did not increase deposition of calcium by detectable amounts. The concentration of calcium was higher in osteogenic media (0.20g/L) than for the control media (0.15g/L), and this could be a factor affecting calcium deposition. Another potential reason for lack of calcium deposition is that 12 days culture was not long enough for PCA to induce calcium deposition.

Recommendations

Assess potential for PCA to induce osteogenic differentiation in mesenchymal stem cells. Positive results could broaden clinical applications to include fracture healing, new bone formation, bone tissue engineering, and regenerative medicine.

Materials and Methods

Cells: Primary human osteoblasts were obtained from Cell Applications, San Diego, CA.

Cell culture: Osteoblasts were expanded in culture using the protocol provided by Cell Applications. Human Osteoblast Basal Media was supplemented with Human Osteoblast Growth Supplement (Cell Applications) containing 0.15g/L calcium. Human Osteoblast Differentiation Media was purchased from Cell Applications, containing 0.20g/L calcium.

Treatment: Osteoblasts were cultured in in Human Osteoblast Basal Media and exposed to Protocatechuic acid (PCA) for up to 12 days at concentrations ranging from 1µmolar to 100µmolar. Osteoblasts were cultured without any PCA as negative control. Osteoblasts were cultured without any PCA in osteogenic media (Human Osteoblast Differentiation Media) as positive control (osteogenic media induces osteogenesis in osteoblasts). Each experiment was conducted in triplicate. Media were changed every 3 days.

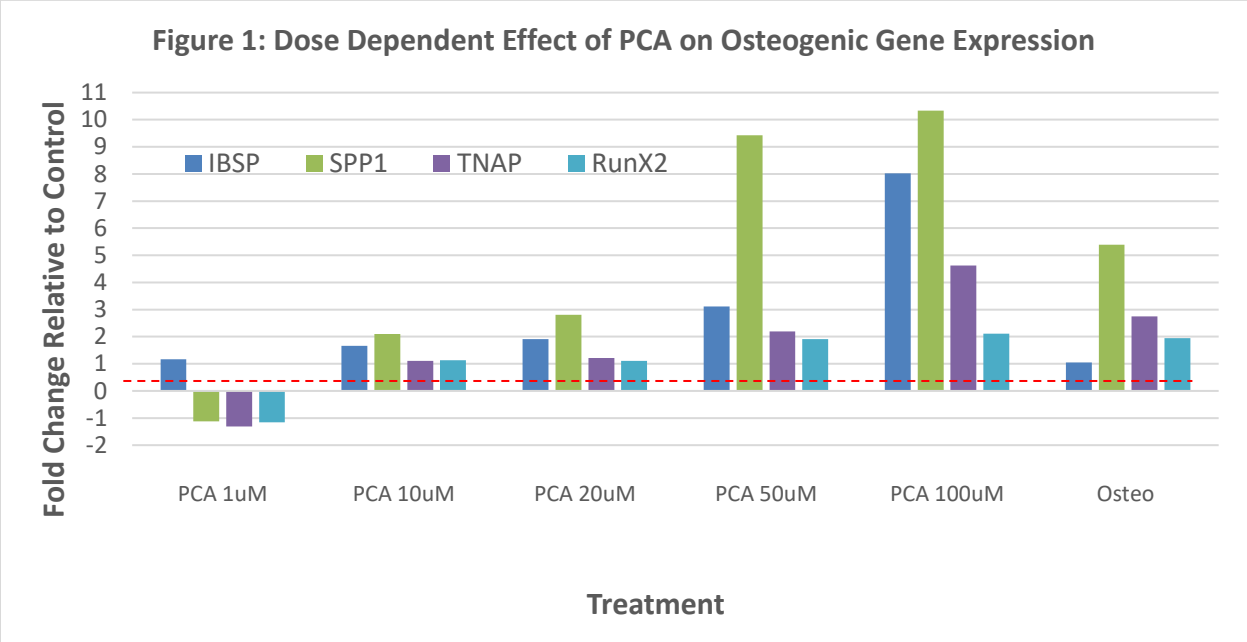
Gene expression: Pro-osteogenic and anti-osteogenic gene expression was measured using qPCR on mRNA extracted from osteoblast cultures. Gene expression was computed as fold-change relative to control group (osteoblasts cultured without any PCA).

Calcium deposition: Osteoblast cultures were tested for calcium deposition using Alizarin red stain.

Results

Pro-osteogenic gene expression

PCA had a dose-dependent effect on all osteogenic genes tested (Figure 1). The largest effect were seen at the highest dose (100µmolar) on the expression of IBSP and SPP1. At this dose, the expression of osteogenic genes was even higher than that measured in the positive control (osteoblasts cultured without any PCA in osteogenic media).



Bone sialoprotein (IBSP) is a major structural protein of bone matrix. This protein binds to calcium and hydroxyapatite and constitutes approximately 12% of the noncollagenous proteins in human bone.

Osteopontin (SPP1) is involved in the attachment of osteoclasts to the mineralized bone matrix.

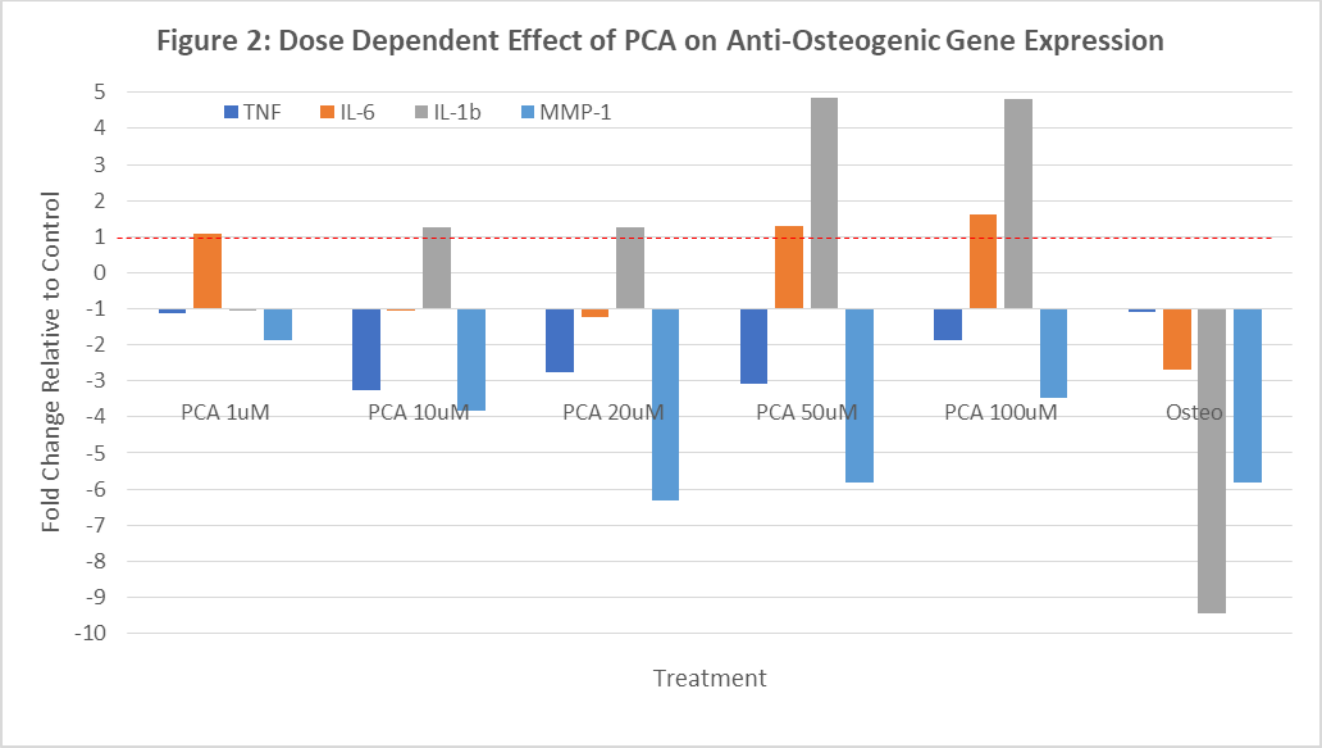
Alkaline phosphatase (TNAP) is essential for the process of mineralization, in which minerals such as calcium and phosphorus are deposited in developing bones and teeth.

RUNX2 acts as a "master switch," regulating a number of other genes involved

in the development of cells that build bones (osteoblasts)

Anti-osteogenic gene expression

PCA had a mixed effect on anti-osteogenic genes, suppressing TNF-α and MMP-1, but increasing IL-1β and IL-6 (Figure 2).



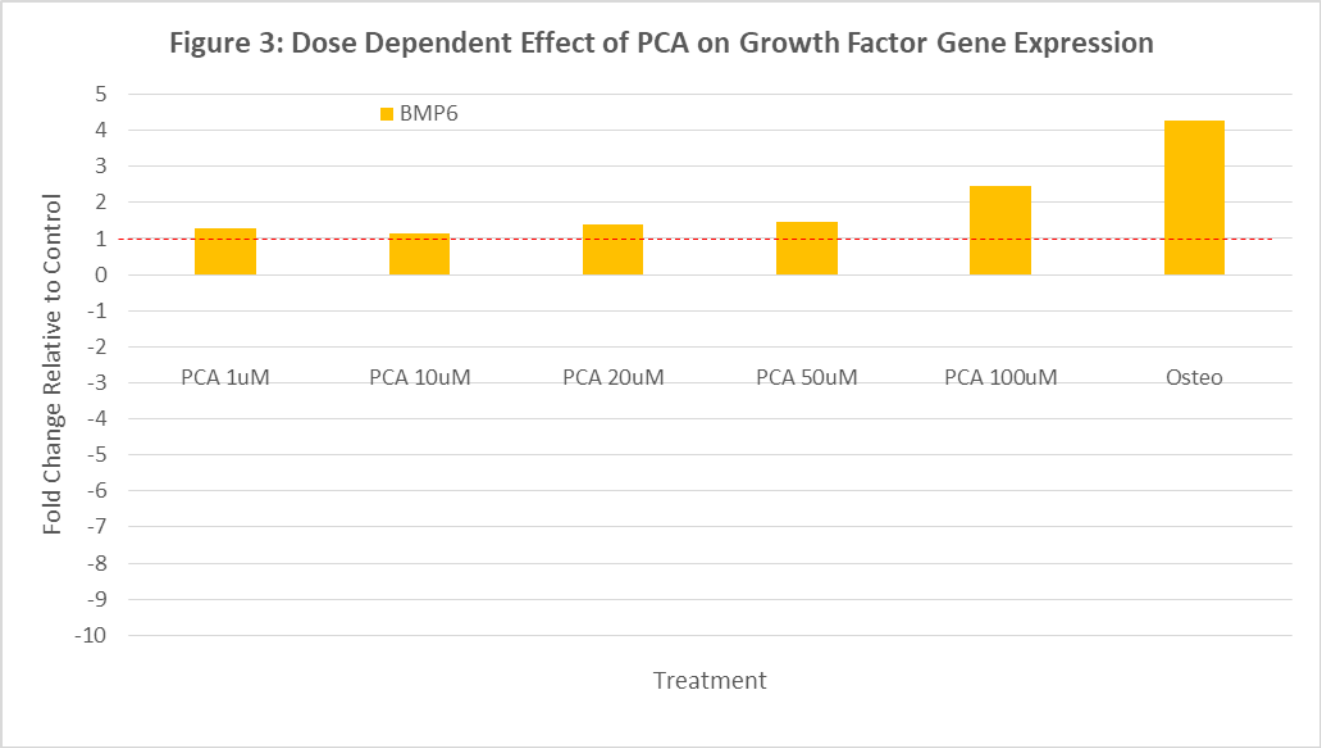
Matrix metalloproteinase 1 (MMP-1) breaks down collagens and in involved in bone resorption.

Tumor necrosis factor-α (TNF-α) triggers inflammation and induces cell death.

Interleukin 6 (IL-6) and Interleukin 1β (IL-β) are involved in inflammation.

Growth factor gene expression

PCA also had a dose-dependent effect on BMP-6 expression. At 100µmolar, BMP-6 rose greater than 2-fold relative to negative control (Figure 3).



Bone morphogenetic protein 6 (BMP-6) binds various TGF-beta receptors and regulates bone development.

Calcium deposition

PCA did not increase deposition of calcium by detectable amounts. The positive control (osteoblasts cultured in osteogenic media) did show evidence of calcium deposition. The concentration of calcium was higher in osteogenic media (0.20g/L) than for the control media (0.15g/L), and this could be a factor affecting calcium deposition. Another potential reason for lack of calcium deposition is that 12 days culture was not long enough for PCA to induce calcium deposition.