ASSESSMENT OF OSTEOGENIC EFFECT OF PROTOCATECHUIC ACID ON HUMAN MESENCHYMAL STEM CELLS

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Objective

This is a proof-of-concept study to determine if Protocatechuic acid enhances osteogenesis in human mesenchymal stem cells (MSC).

Report Summary

Protocatechuic acid (PCA) increased expression of important osteogenic genes in mesenchymal stem cells. PCA increased Osteopontin and RUNX2 expression even greater than the osteogenic conditions. PCA increased expression of alkaline phosphatase compared to control but not as much as osteogenic conditions. PCA induced deposition of calcium in at least one cell line by 3 weeks.

Recommendations

This study provided evidence that PCA induced expression of osteogenic genes in mesenchymal stem cells. These results, if clinically validated, could broaden clinical applications to enhance fracture healing, new bone formation, bone tissue engineering, and regenerative medicine.

Materials and Methods

Cells: Primary human mesenchymal stem cells (MSC) were obtained from RoosterBio Inc., Frederick, MD; ATCC, Manassas, VA; and Cell Applications, San Diego, CA.

Cell culture: MSC were expanded in culture, using the protocol provided by the respective suppliers, before being subjected to the experimental conditions.

Treatment: MSC were cultured in supplier-provided culture media and exposed to Protocatechuic acid (PCA) for 14 or 21 days at concentrations ranging from 200µmolar to 2000µmolar (2mM). MSC were cultured without any PCA as negative control. To serve as positive control, MSC were cultured without any PCA in osteogenic media (Human Osteoblast Differentiation Media, Cell Applications, Catalog number: 417D-250). Osteogenic media is commonly used to induce osteogenesis. Each experiment was conducted in triplicate. Media was changed twice a week.

Gene expression: Osteogenic gene expression was measured using qPCR on mRNA extracted from MSC cultures. Gene expression was computed as fold-change relative to the control group (MSC cultured without any PCA). MSC cultured in osteogenic media was used as positive control as this protocol is commonly used to induce osteogenesis.

Calcium deposition: MSC cultures were tested for calcium deposition using Alizarin red stain at 21 days.

Results

Osteogenic Gene Expression

1. PCA had positive effect on three of the four osteogenic genes tested (Figures 1 - 4). The largest effect was noted in the expression of Osteopontin (Figure 1), which plays a critical role in the bone repair and maintenance. At all doses and in all three cell lines, the expression of Osteopontin was even higher than that measured in the positive control (MSC cultured without any PCA in osteogenic media).
2. PCA also increased RUNX2 at most dose levels. Overall RUNX2 expression was higher at 2 weeks than at 3 weeks. RUNX2 expression was mostly higher than the positive control.
3. PCA increased the expression of alkaline phosphatase relative to negative control but not greater than the osteogenic conditions.
4. PCA did not increase the expression of bone sialoprotein relative to either negative or positive controls.

The above results were broadly similar in all three MSC lines.

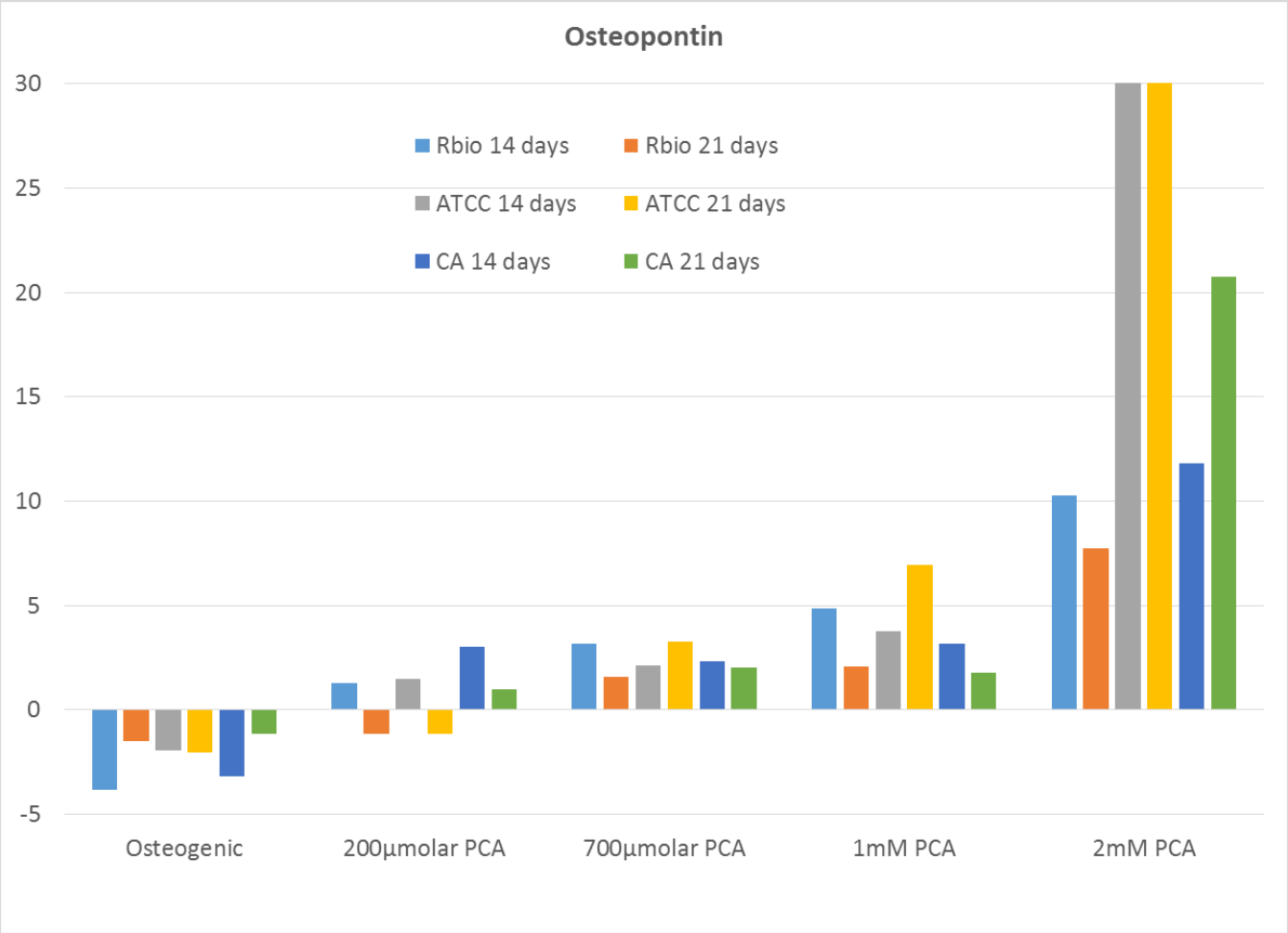

Figure 1: At all doses and in all three cell lines, the expression of Osteopontin was even higher than that measured in the positive control.

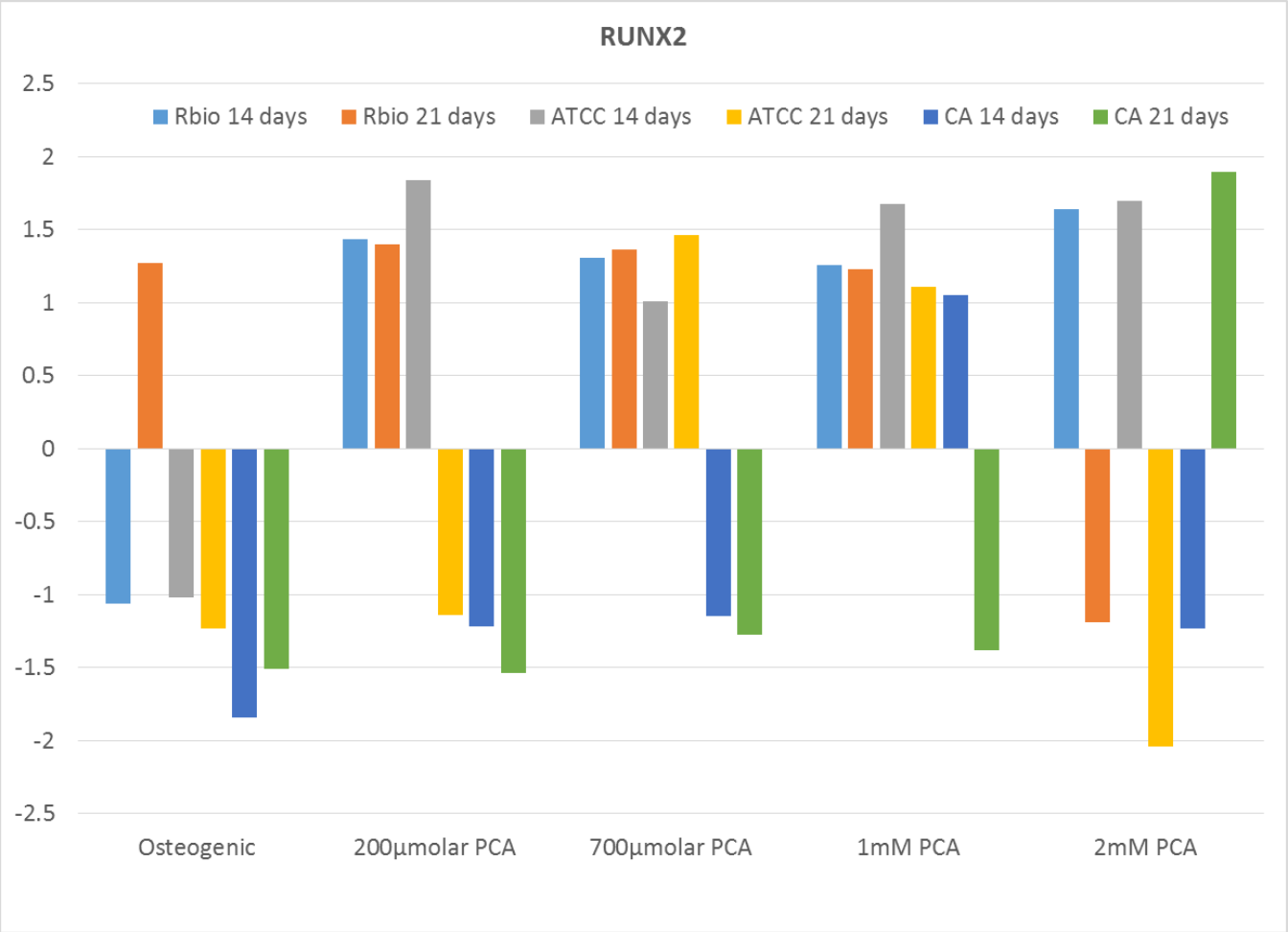

Figure 2: PCA increased RUNX2 expression relative to negative control and was generally equivalent to or higher than the positive osteogenic control.

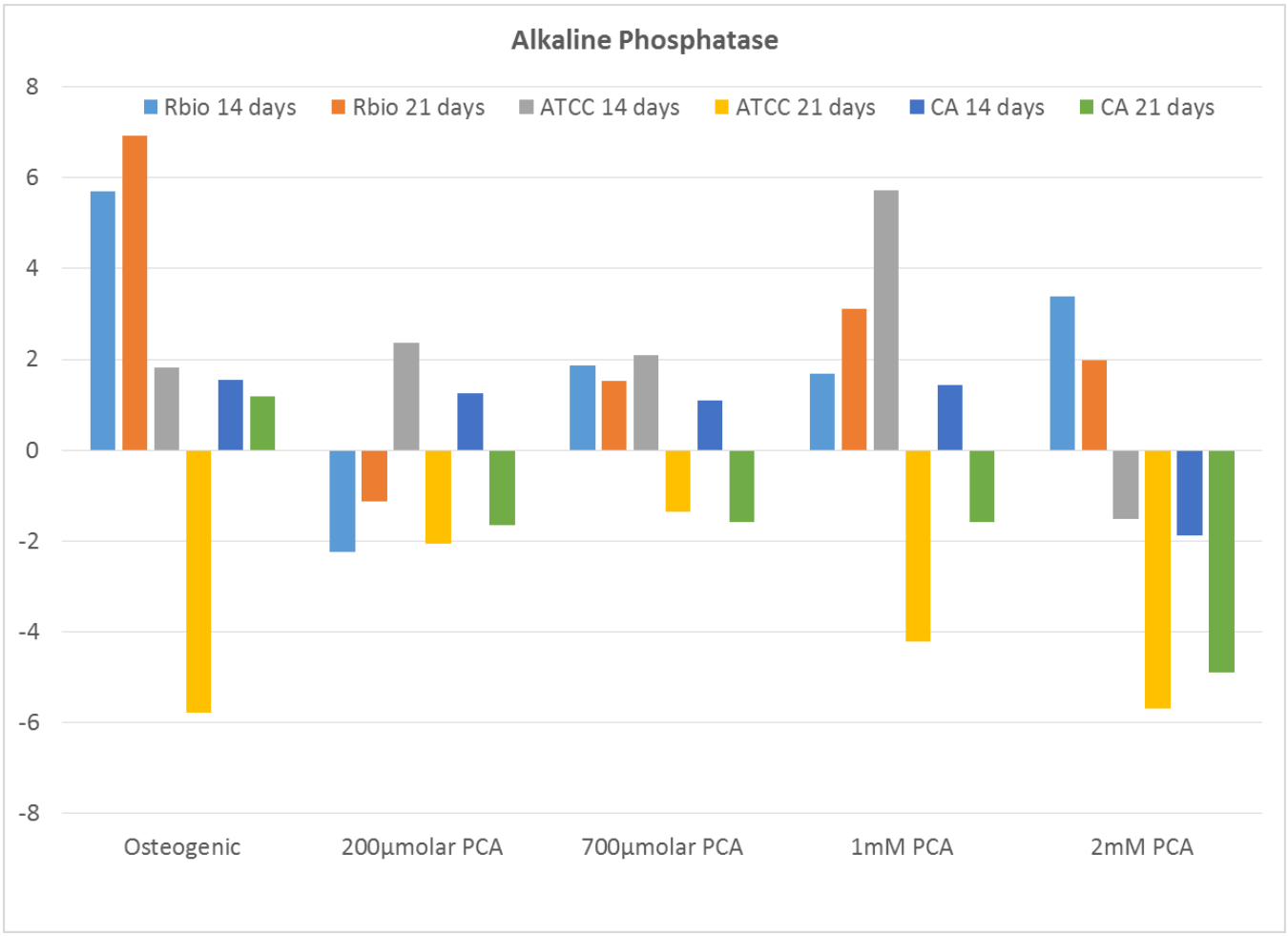

Figure 3: PCA increased the expression of alkaline phosphatase relative to negative control in the majority of dose levels and time points but not relative to the osteogenic conditions.

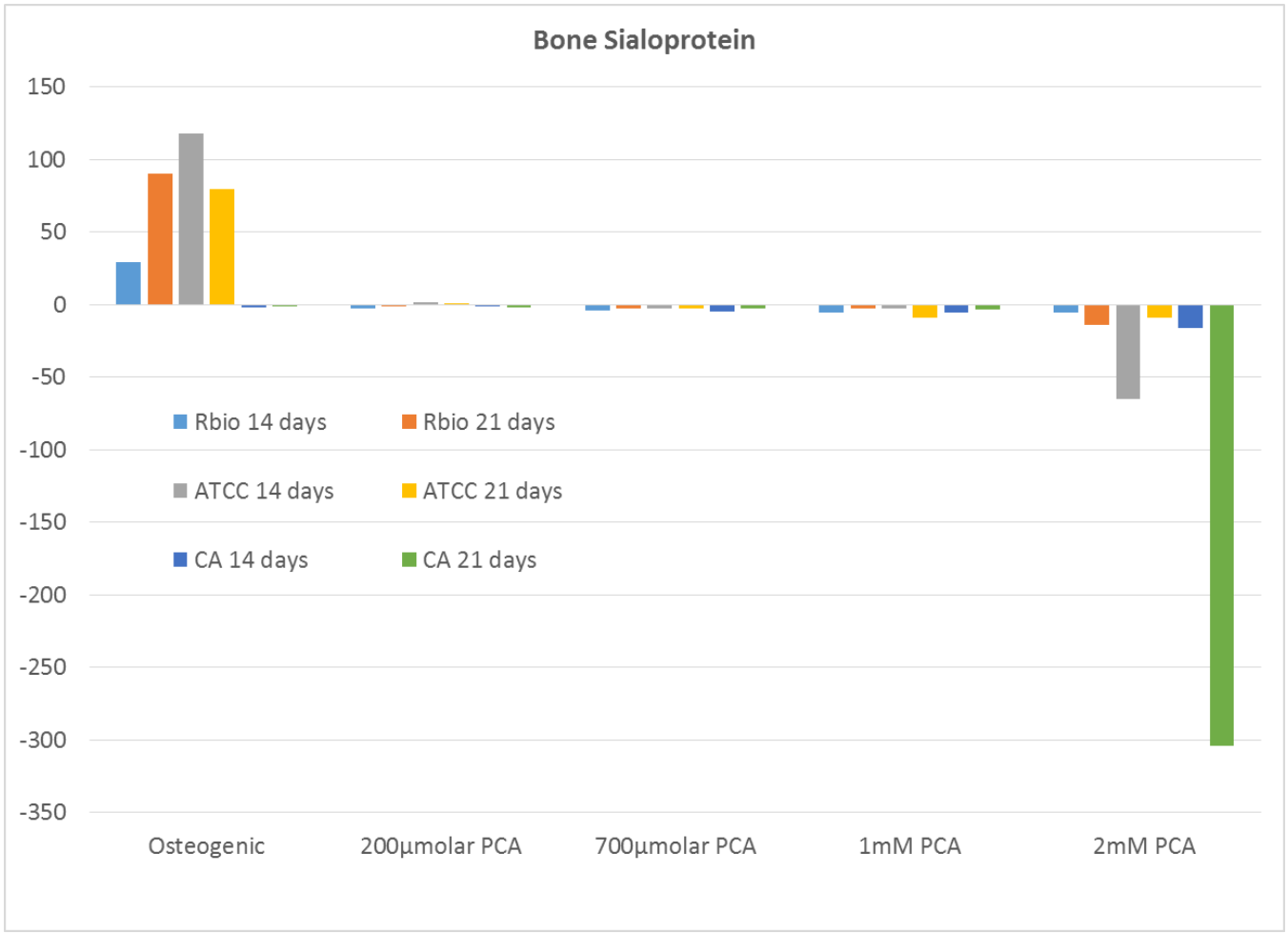

Figure 4: PCA had no significant positive effect on bone sialoprotein.

Calcium Deposition

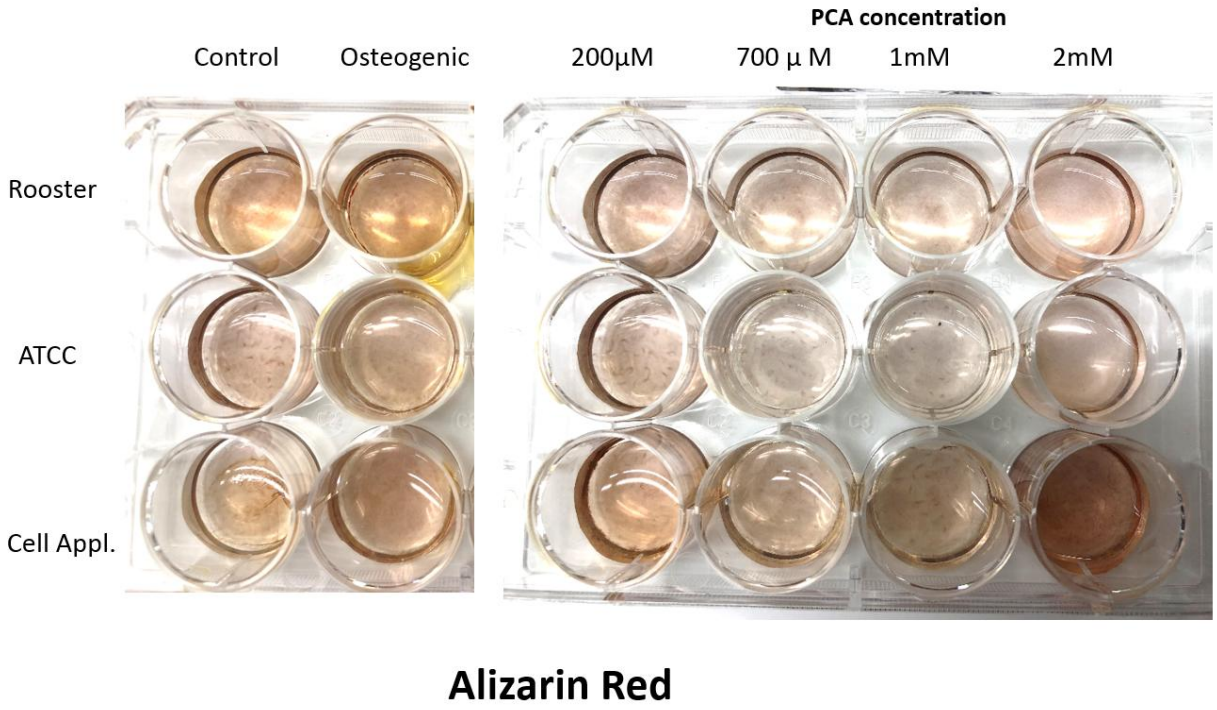

Figure 5: PCA visibly increased the deposition of calcium in one of the three cell lines (Cell Applications)

Discussion

As expected, there was some variability in the osteogenic response from MSC from different donors. Overall, PCA had a positive effect on three of the four important osteogenic genes. PCA also increased calcium deposition in one of the three cell lines tested. Possible reasons could be donor-to-donor variability or that the effect on calcium deposition, which occurs later in the osteogenic timeline, could be more apparent after 3 weeks.

Osteopontin plays a critical role in the maintenance of bone, especially as a molecule involved in the response of bones to external stress. It is also involved in other homeostatic defense mechanisms in the mammalian organism. Osteopontin is expressed in cells of the osteoblastic lineage, and possibly those including osteocytes, which are exposed to mechanical stress.
RUNX2 acts as a "master switch," regulating a number of other genes involved in the development of cells that build bones (osteoblasts). RUNX2 tends to be expressed early and coordinates the expression of other osteogenic genes.
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.

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.