THE CALCIMIMETIC CALINDOL PREVENTS HIGH PHOSPHATE-INDUCED VASCULAR CALCIFICATION BY UPREGULATING MATRIX GLA PROTEIN

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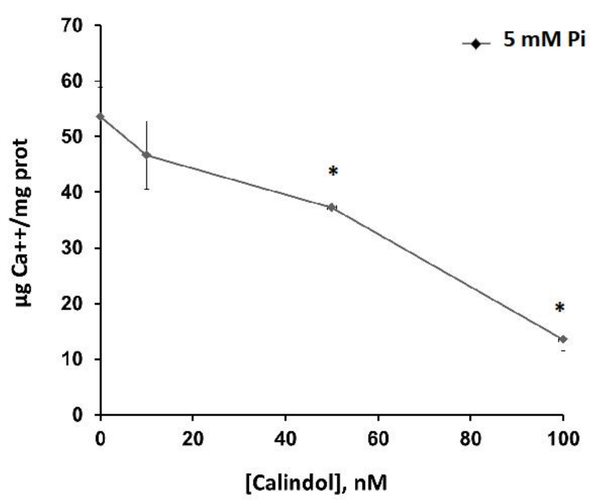
Background and Aim

- Calcimimetics are used to control secondary hyperparathyroidism and, potentially, to delay the progression of VC in dialysis patients
- VC, induced by high-phosphate and uremic milieu, is characterized by a passive deposition of calcium-phosphate (Ca-Pi) and an active transformation of vascular smooth muscle cells (VSMCs) in osteoblastic-like cells
- Several proteins promote or inhibit the phenotypical change of VSMCs towards osteoblastic-like cells.
- The aim of these *in vitro* studies was to investigate the direct effects of the calcimimetic calindol on the progression of high Pi-induced VC High serum phosphate (Pi) levels represent a major issue in dialysis patients, because associate with secondary hyperparathyroidism, vascular calcification (VC), and cardiovascular outcomes

Materials & Methods

- Rat VSMCs were cultured and challenged with inorganic Pi (5mM) to induce calcification. (Calcification medium: DMEM high glucose,12% FBS, 10 mM sodium pyruvate, 100 U ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin and AA). Calindol were added 3 hours before Pi
- Ca deposition was evaluated by an histological analysis (Alizarin Red staining) and quantified by colorimetrical method (*o*-cresolphthaleine complexone) at day 7 of calcification
- α-actin, Smooth Muscle 22a (SM22a) and Axl protein content was analyzed by western blot separating 5, 1 or 50 mg of total protein by electrophoresis on SDS-polyacrylamide gel and transferred to PVDF membrane
- Total RNA was extracted and Bone Morphogenic Protein-2 (BMP-2), Core Binding Factor alpha-1 (Cbfa-1/RUNX2) and MGP mRNA expression was evaluated by TaqMan PCR using rat β-actin housekeeping gene

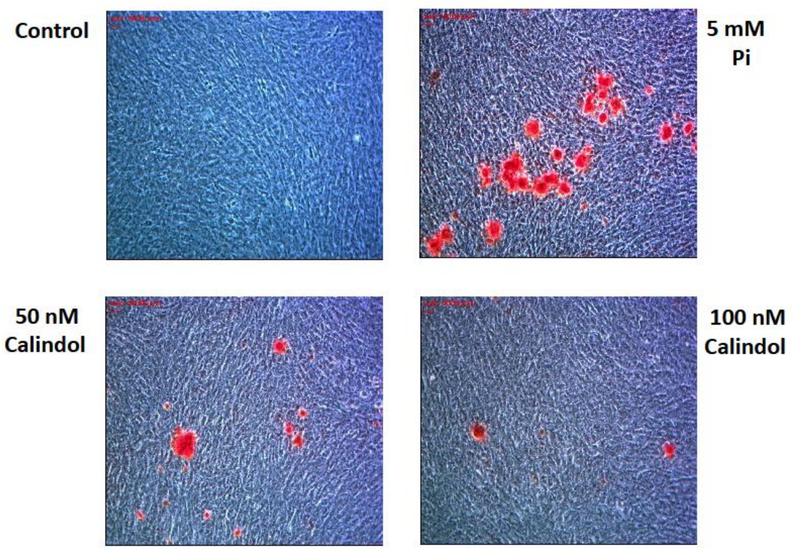
Increasing Concentrations of Calindol Prevent Calcium Deposition in VSMCs



*p<0.01 *vs* Pi

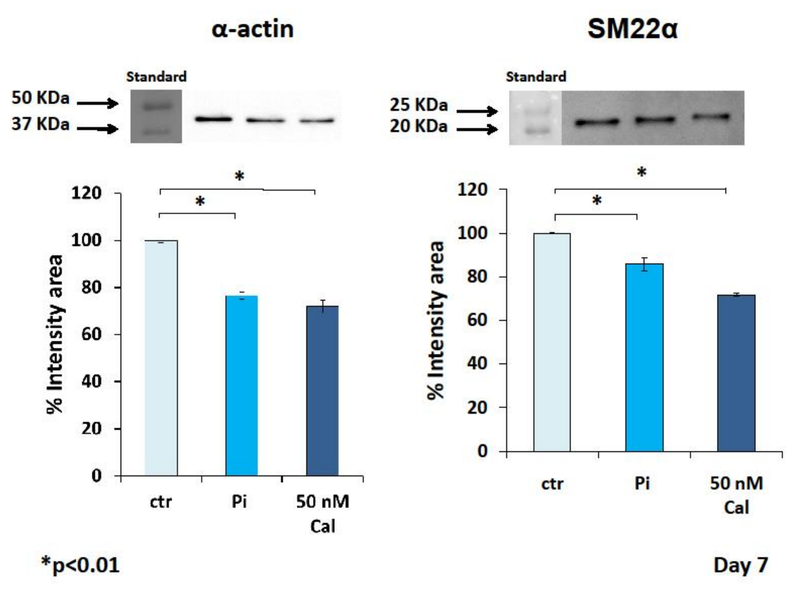
Day 7

Effect of Calindol on Calcium Deposition



Day 7

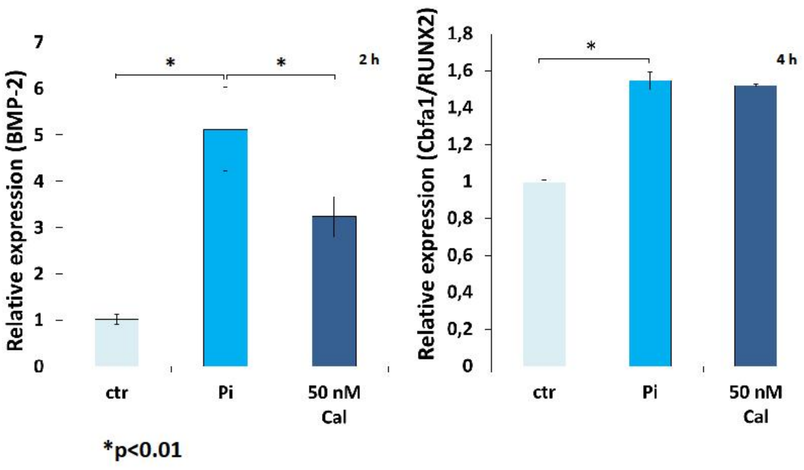
Calindol Does Not Prevent VSMCs Lineage Markers from Pi-induced Downregulation



*p<0.01

Day 7

Calindol Reduces High Pi-induced BMP-2 Upregulation, with no Effects on Cbfα1/RUNX2 mRNA Levels



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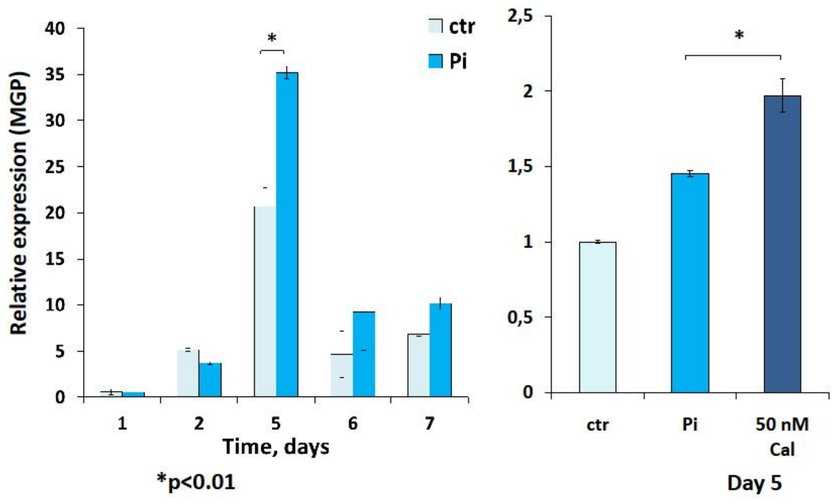
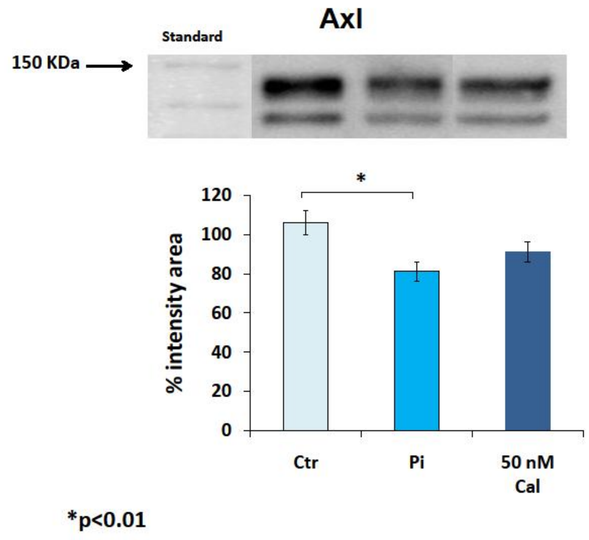
Calindol Induces a Significant Increase in MGP mRNA Levels After Pi Challenge



*p<0.01

Day 5

Calindol Does Not Affect Pi-induced Axl Downregulation



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Day 7

Conclusions

- We demonstrated that the calcimimetic calindol increases MGP gene expression in rat VSMCs
- The inhibitory effect of calindol on VC is probably due to its stimulatory role on the Calcium-Sensing Receptor (CaSR), leading to an increase in the synthesis of MGP by VSMCs
- The increase of MGP in VSMCs pre-treated with calindol may be interpreted as a defensive mechanism against vascular calcification
- Furthermore, calindol reduces high Pi-induced BMP-2 upregulation, with no effects on Cbfα1/RUNX2 mRNA levels, on VSMCs lineage markers expression and on apoptosis

References

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- Ciceri P, Elli F, Brenna I, Volpi E, Brancaccio D, Cozzolino M. *The calcimimetic Calindol prevents high phosphate-induced vascular calcification by upregulating matrix GLA protein.* Nephron Exp Nephrol. 2013 Mar 28;122 (3-4):75-82.

50ERA-EDTA

525--MP

CKD-MBD - A

mario cozzolino

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