A NEW *"IN VITRO"* MODEL TO DELAY HIGH-PHOSPHATE INDUCED VASCULAR CALCIFICATION PROGRESSION

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Background and Aim

• Vascular calcification (VC) is a relevant complication in chronic kidney disease leading to increase in cardiovascular disease mortality.

• VC, induced by high-phosphate and uremic milieu, is characterized by a passive deposition of calcium-phosphate (Ca-P) and an active transformation of vascular smooth muscle cells (VSMCs) in osteoblastic-like cells.

•Recently, it has been demonstrated that longer nocturnal haemodialysis have a beneficial impact on reducing the progression of VC in CKD patients, through a better control of serum Pi levels

•The present study had the purpose to try to mimic *in vitro*, the modification of Pi concentration that occurs in CKD patients, in order to study its effect on VSMC calcification.

Materials & Methods

• Rat VSMCs were cultured and challenged with inorganic phosphate (Pi) to induce calcification. (Calcification medium: DMEM high glucose,15% FBS, 10 mM sodium pyruvate, 100 U ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin and AA). Medium was replaced every 2 or 3 days and we define intermittent suspension (IS) the temporary suspension of high Pi every time medium was replaced followed by the re-challenge with 5mM Pi after the IS period up to 11 days.

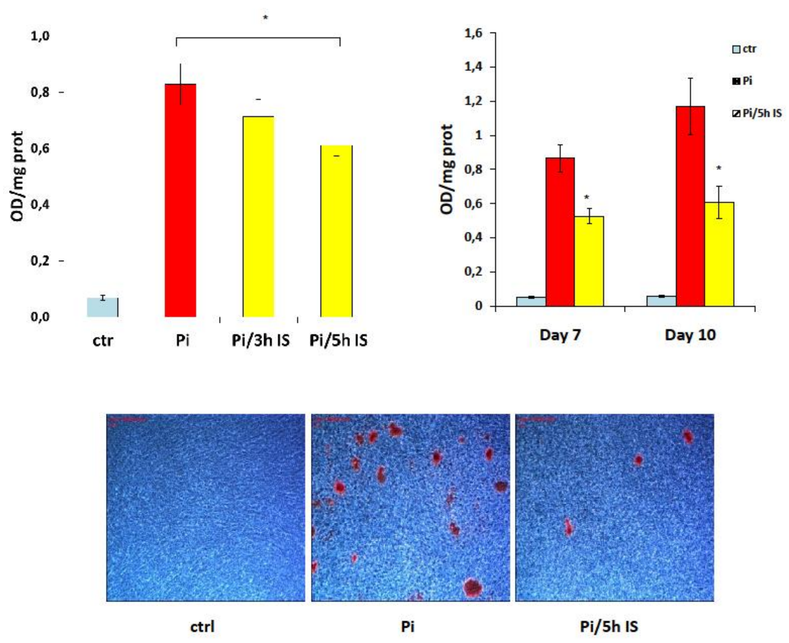
• Calcium (Ca) deposition was evaluated by histological analysis (Alizarin Red staining) and quantified colorimetrically by destaining.

• α-actin, SM22α, Axl and RUNX2 protein content was analyzed by western blot. Total RNA was extracted and BMP-2 mRNA expression was evaluated by TaqMan PCR using rat β-actin as housekeeping gene.

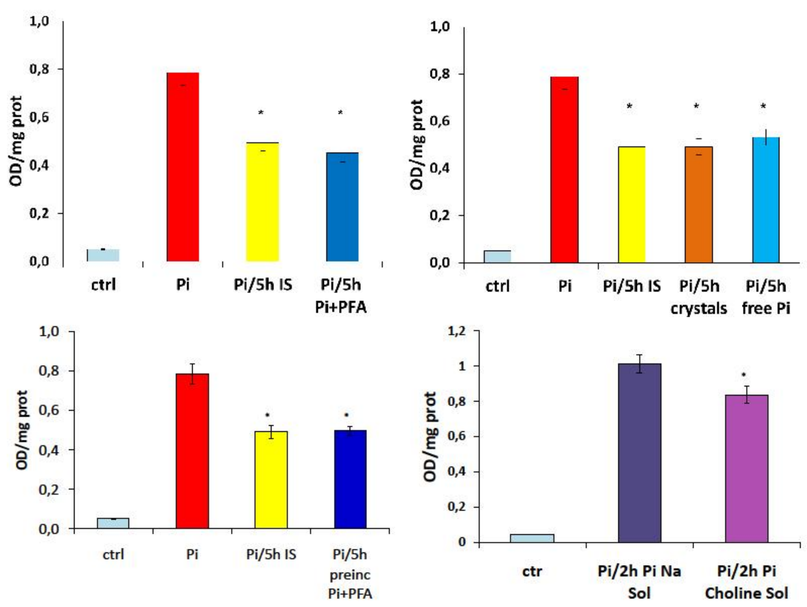
•Semithin (0,5µm) sections were stained with toluidine blue and ultrathin sections (50-60nm) were counterstained with uranyl acetate and lead citrate, to be observed in a electron microscope.

•The immunohistochemical evaluation of osteonectin protein was performed using monoclonal antibody to osteonectin (N50 1:30.000).

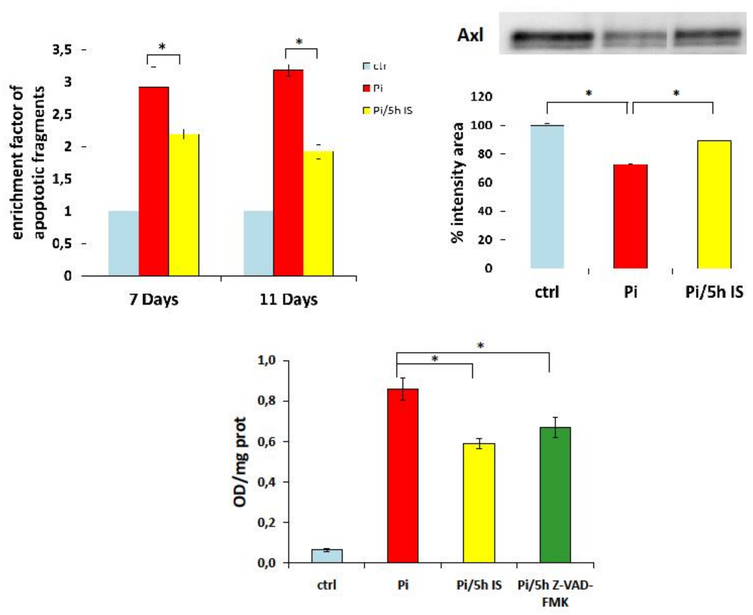
High Phosphate Intermittent Suspension (IS) Inhibits Vascular Calcification Time Dependently



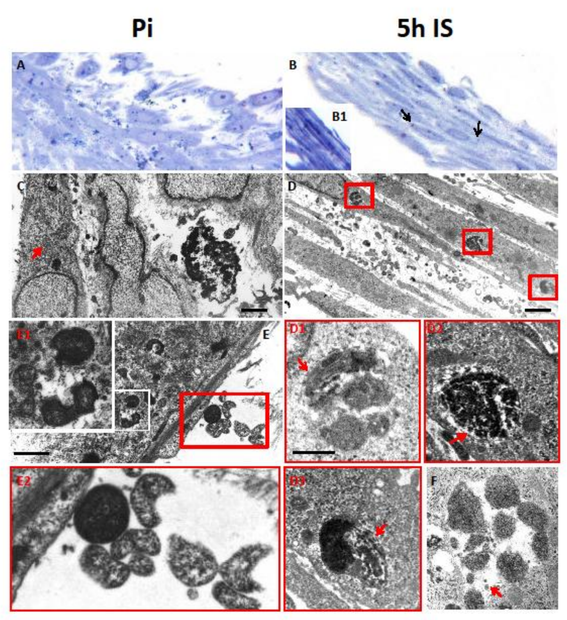
Both CaP Crystals and Free Pi IS Delay Calcification to the Same Extend of Total Pi IS



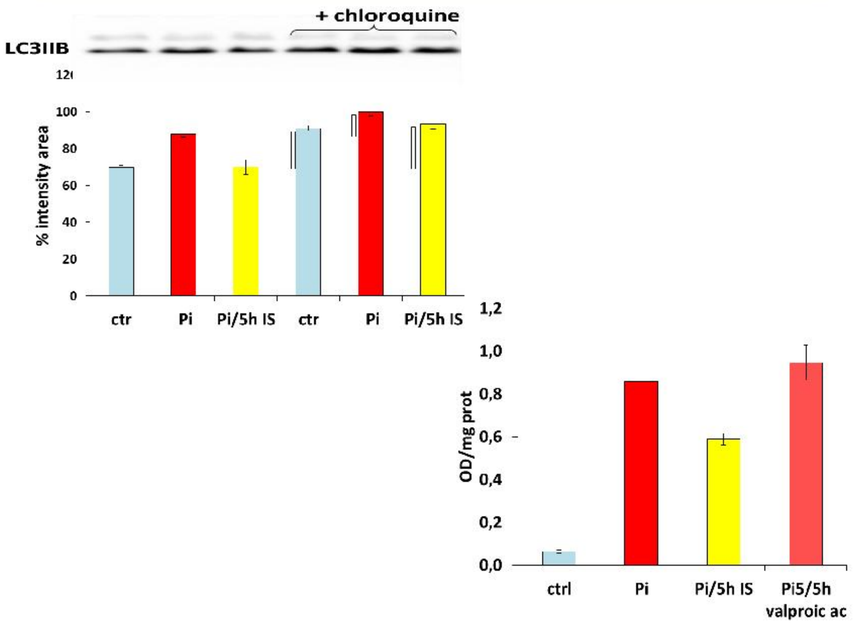
IS Prevents High Pi Calcification by Preventing Apoptosis



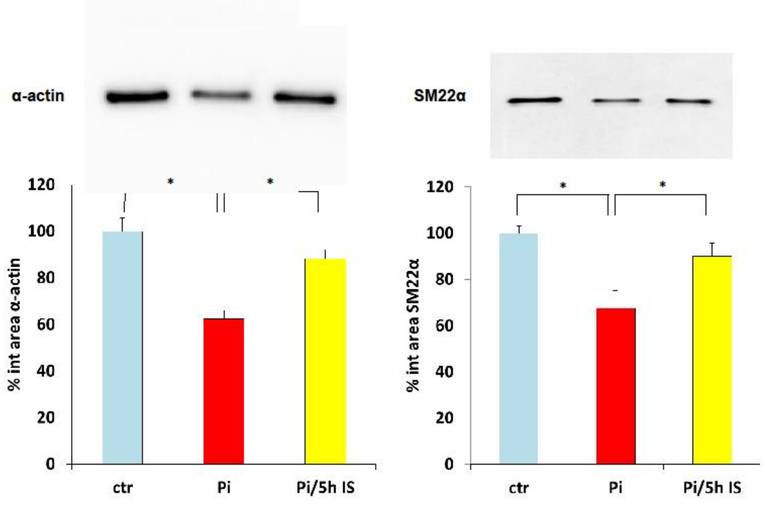
IS Induces Autophagosomic Structure Partially Preventing Mitochondria Calcification



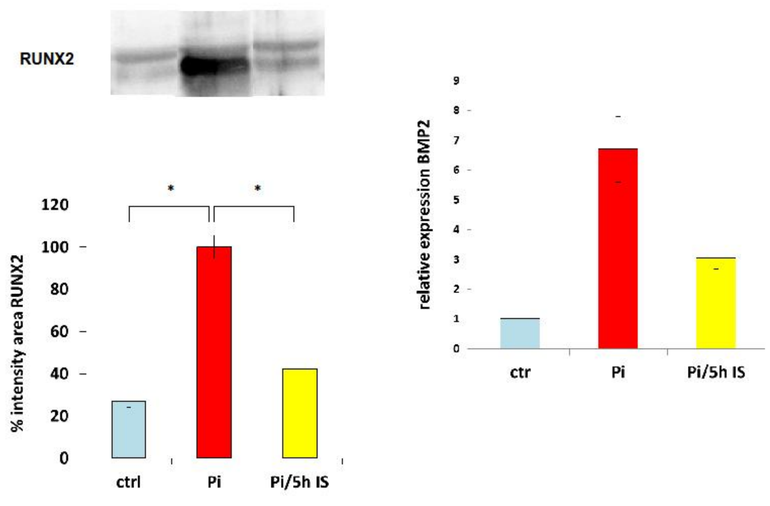
IS Potentiates Autophagy but Its Modulation does not Affect Calcification



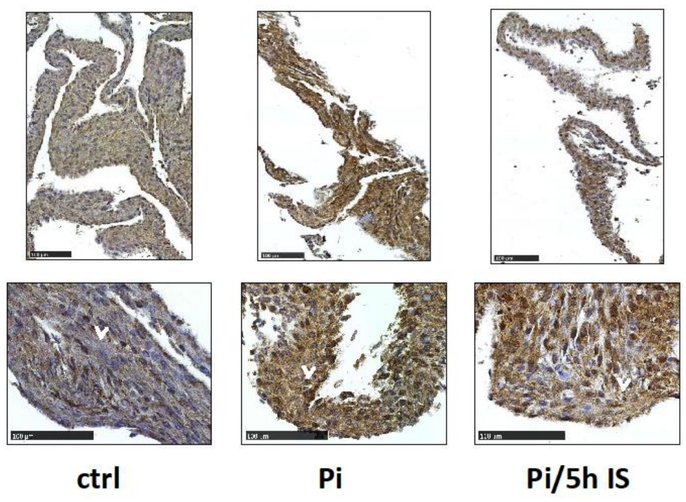
IS Prevents High Pi Loss of SM-Markers Protein Expression



IS Prevents High Pi RUNX-2 and BMP-2 Induction



IS Prevents High Pi Induction of Osteonectin Protein Expression



Conclusions

•High Pi (Intermittent Suspension) IS was able to induce a significant inhibition of high Pi calcification, maximal at 5 hours.

•Interestingly, the delay in calcification is a consequence of either the absence of free Pi or CaP crystals being comparable to the total effect obtained during the 5h-IS.

•The protective effect of IS was mediated by the reduction of apoptosis.

•Autophagy, during IS, was potentiated by increasing the autophagic flux, but its inhibition with valproic acid did not affect calcification.

•IS prevented VSMC osteoblastic differentiation by preserving smooth muscle lineage markers expression.

•From these in vitro data it seems that to delay significantly VC is necessary and sufficient the IS of high Pi challenge. The IS was able to prevent significantly apoptosis, to induce a potentiation in autophagy, and to prevent osteoblastic differentiation.

References

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