IRON CITRATE REDUCES HIGH PHOSPHATE-INDUCED VASCULAR CALCIFICATION BY INHIBITING APOPTOSIS

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Background and Aim

• Chronic kidney disease (CKD) is strongly associated with increased cardiovascular (CV) risk and mortality.

•One of the main features of the increased CV risk is vascular calcification (VC) that in CKD patients is mainly medial calcification and one of the strongest VC inducers in CKD patients is phosphate (P).

•To control P levels in CKD are utilized P binders, and recently two iron-based P binders are available for the clinical practice.

•Since anemia is a well characterized medical problem in CKD patients that requires iron dietary supplementation, the use of iron-based P binders can control hyperphosphataemia having also an effect on iron levels.

•Lacking data on effect of iron on the progression of VC in CKD patients, the aim of this project is to study the action of iron on high-P induced VC.

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.

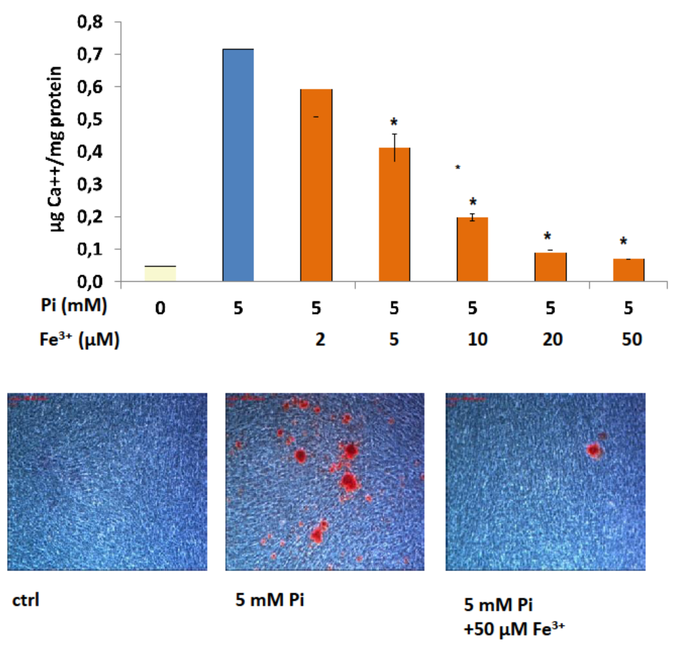
•Calcium (Ca) deposition was evaluated by histological analysis (Alizarin Red staining) and quantified colorimetrically by destaining.

• α-actin, SM22α, GAS6 and Axl protein content was analyzed by western blot. Total RNA was extracted and RUNX2 and BMP-2 mRNA expression was evaluated by TaqMan PCR using rat β-actin as housekeeping gene.

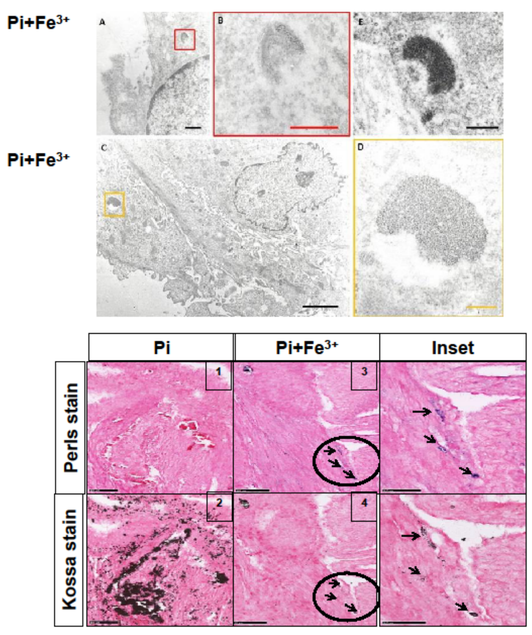
•Semithin (0,5µm) sections were stained with toluidine blue and ultrathin sections (50-60nm) were counterstained with uranyl acetate and lead citrate, to be observed in a electron microscope.

•VSMC morphology was assessed with hematoxylin and eosin staining; Perls' Prussian Blue and Von Kossa was performed to visualize iron and Ca deposits. The immunohistochemical evaluation of α-actin protein was performed using monoclonal antibody to α-actin (1A4, dil. 1:100).

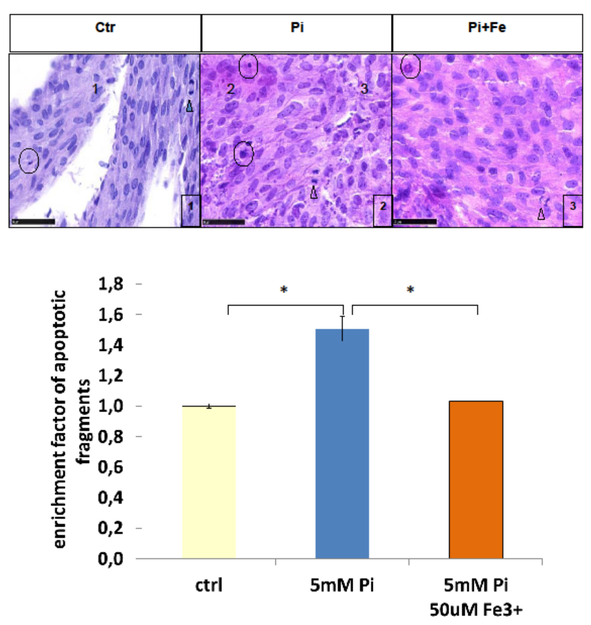
Ferric Citrate Prevents High-Pi Induced Extracellular Calcium Deposition



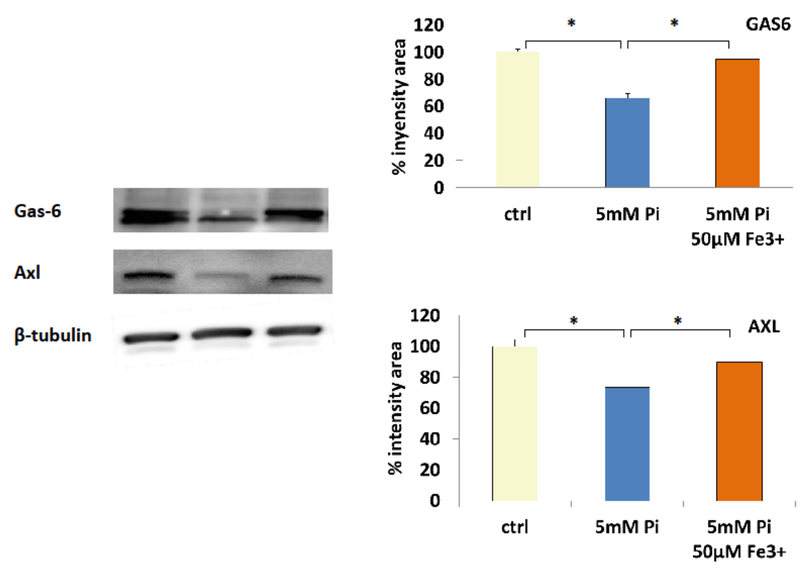
Treatment with Ferric Citrate Induces Iron Accumulation and Deposition



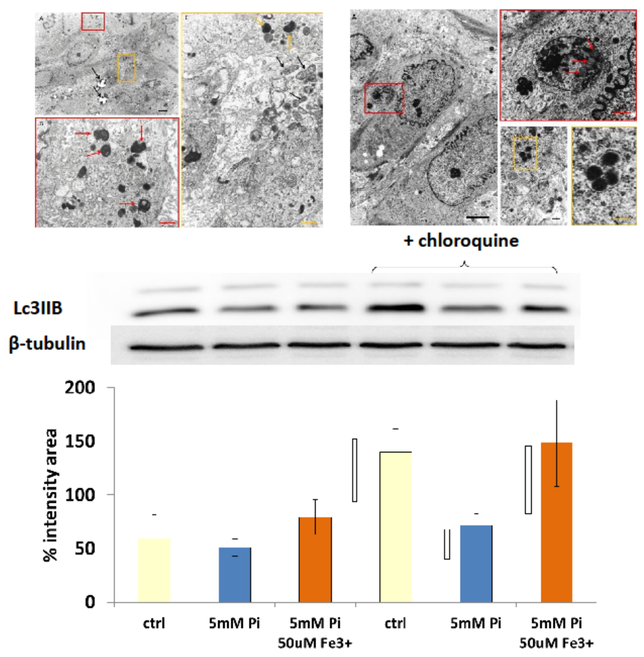
Ferric Citrate Prevents High-Pi Induced Apoptosis



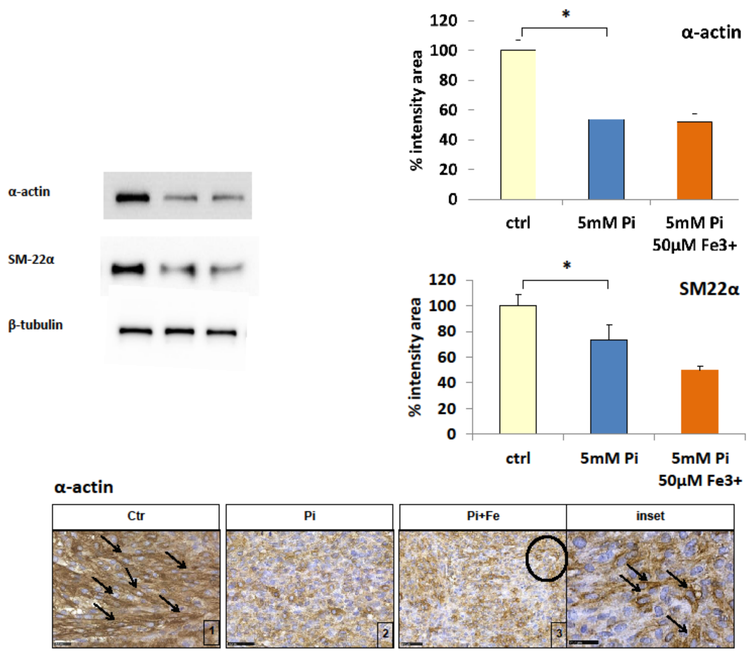
Ferric Citrate Prevents High-Pi Induced Down-regulation of the Pro-survival Pathway Gas6/Axl



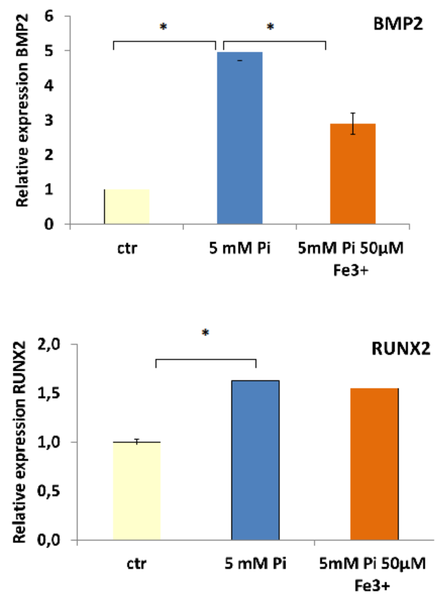
Ferric Citrate Potentiates Autophagy



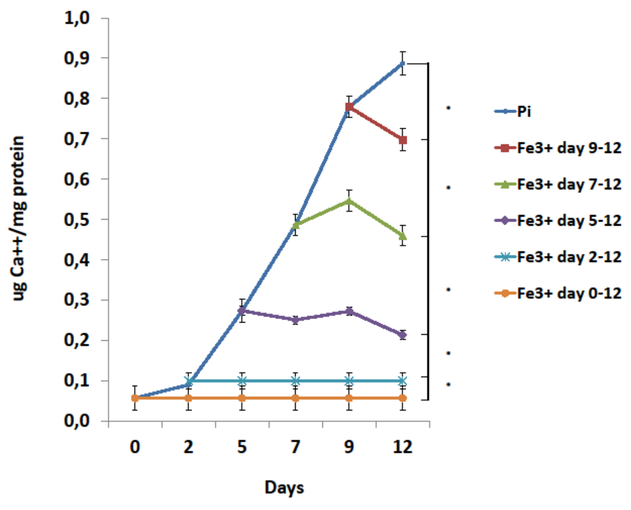
Ferric Citrate does not Prevent High-Phosphate SM Lineage Markers Down-regulation



Ferric Citrate Prevents High Pi BMP-2 Induction but does not Affect RUNX2



Therapeutic Administration of Ferric Citrate Blocks High-Pi Calcification



Conclusions

•Ferric Citrate was able to reduce significantly and concentration-dependently high-Pi calcification.

•Iron accumulated in the cells and interestingly was present in Ca deposits.

•The protective effect of iron was mediated by the reduction of apoptosis and by preventing the down-regulation of the pro-survival pathway GAS6/Axl.

•Autophagy was potentiated by iron by increasing the autophagic flux.

•Iron did not affect smooth muscle lineage marker expression, even if from the morphological point of view α-actin had a better structure conservation with a probable better functional activity.

•Iron partially affected osteoblastic markers induction preventing BMP2 increase but not RUNX2 elevation.

•Interestingly, iron administration when calcification was already started completely blocked Ca deposition exacerbation.

•Taken together our data demonstrated a strong effect of iron in preventing and blocking high-Pi induced calcification through prevention of apoptosis and preservation of autophagy.

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Chronic Kidney Disease. Bone disease.
Mario Cozzolino
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21st-24th, 2016
Held jointly with Österreichische Gesellschaft für Nephrologie
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