EFFECT OF IRON ON MATRIX AND SIMIL-OSTEOBLASTIC DIFFERENTIATION IN PHOSPHATE-CHALLENGED VASCULAR SMOOTH MUSCLE CELLS

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Background and Aims

• In vessel walls, vascular smooth muscle cells (VSMCs) are surrounded by a highly structured extracellular matrix (ECM) composed of collagen, elastin, fibronectin and proteoglycans. Glycoproteins, associated to elastic fibers, contributes to VSMCs contractility.

• In Chronic Kidney Disease (CKD) patients, high phosphate (Pi)-exposed VSMCs go through simil-osteoblastic transformation, loss contractility and increase ECM proteins (type I and III collagen, SPARC, fibronectin) deposition, leading to vascular calcification (VC).

• Considering that two iron-based Pi binders are recently utilized for CKD treatment, the aim of this *in vitro* study is to elucidate the direct effect of iron on ECM.

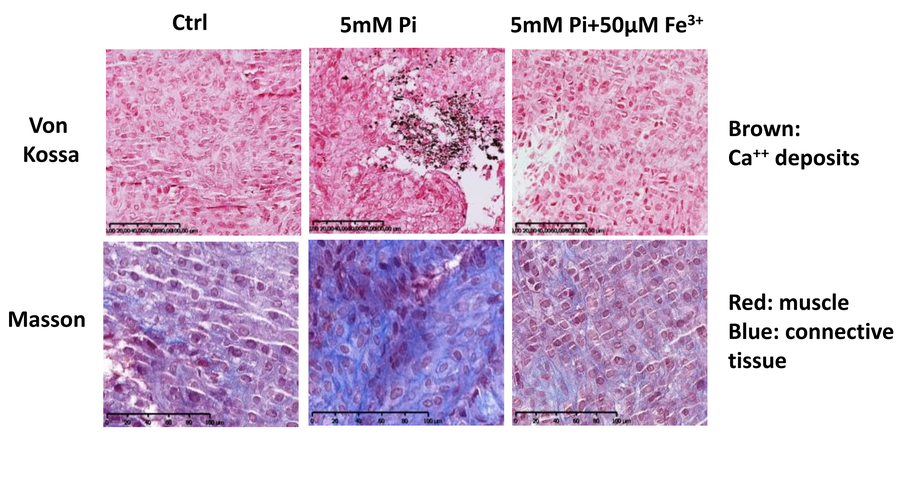
Materials & Methods

• Rat VSMCs were cultured and challenged with inorganic phosphate (Pi) to induce calcification for 7 days. (Calcification medium: DMEM high glucose, 12% FBS, 10 mM sodium pyruvate, 100 U ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin and 50 ug/ml AA). Medium was replaced every 2 or 3 days and some cells received 50 µM iron citrate pretreatment for 7 days .

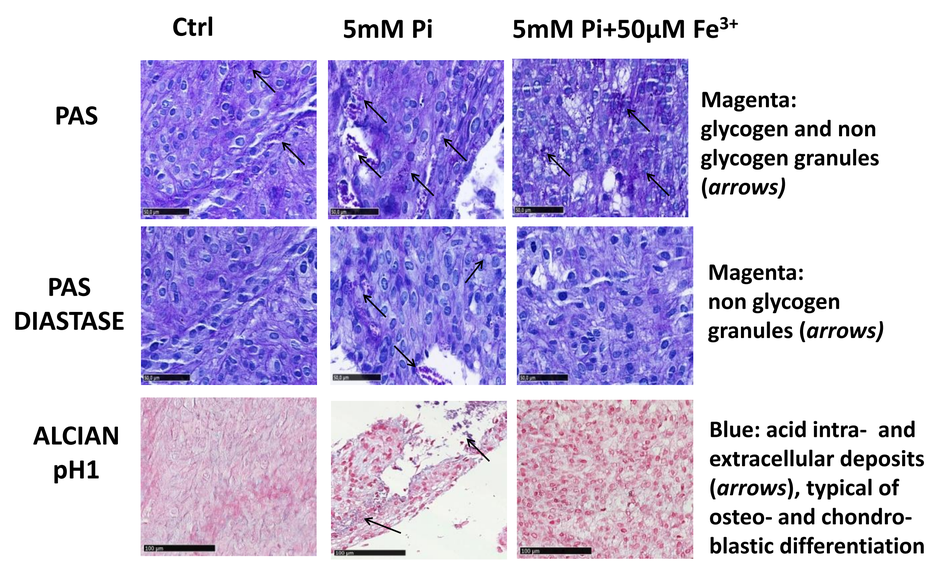
• α-actin protein expression was evaluated by immunohistochemistry and western blot.

• Extracellular Ca^{++} deposition was evaluated by Von Kossa staining. Masson, PAS, PAS-diastase and Alcian Blue pH1 stainings were performed to study the ECM. Each histological sample was evaluated by an arbitrary score index based on number of granules, size and staining intensity (ranging from 0 to 4).

Iron Citrate Inhibits High Pi-induced Ca Deposition and Promotes Muscle Fiber Phenotype

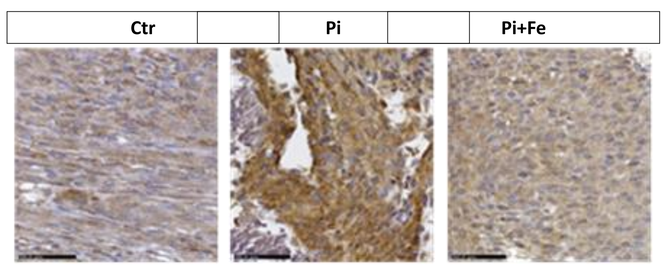


Iron Citrate Preserves the Glycogen and Non Acidic Nature of Matrix Granules, Typical of VSMC

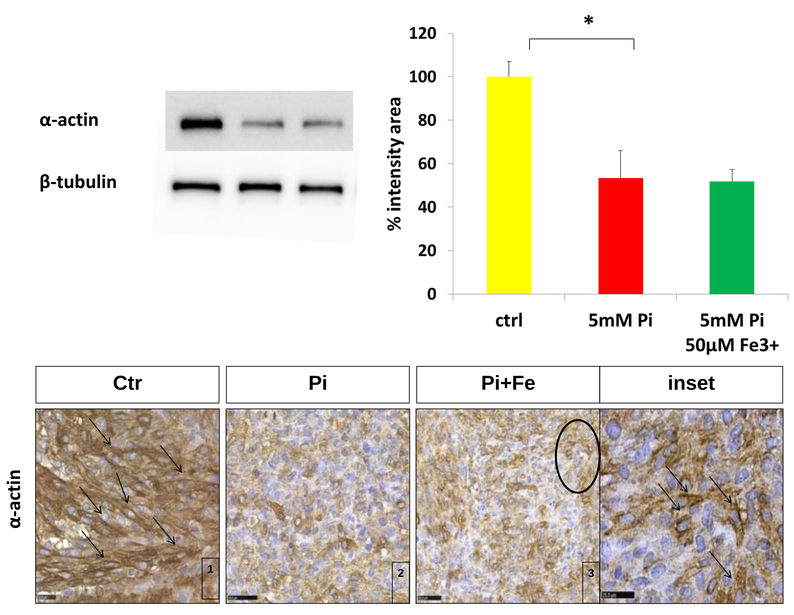


Arbitrary score	Ctr	5mM Pi	5mM Pi +50µM Fe^{3+}
PAS	1,6±0,3	2,8±0,2	2,0±0,3
PAS Diastase	0	2,8±0,4	0
Alcian pH 1	0	2,5±0,3	0

Iron Citrate Affects Osteoblastic Differentiation by Preventing Pi-Induced Osteonectin Induction and by Promoting α-actin Functional Structure



Arbitrary score	Ctr	5mM Pi	5mM Pi +50µM Fe^{3+}
Osteonectin	0,8±0,2	1,9±0,4	0,6±0,2



Conclusions

- Iron citrate prevents high Pi-induced Ca deposition in VSMC.
- High Pi-challenged cells appear rich in connective tissue and collagen fiber, loosing probably part of their contractility properties. Interestingly pretreatment with iron citrate partially preserves muscle fibers and probably elasticity.
- Iron citrate prevents, in high-Pi challenged VSMC, the shift from a glycogen-dependent nature of matrix granule, typical of muscle cells, to a mucins-rich granules phenotype, typical of osteoblasts.
- Iron citrate partially prevents Pi-induced VSMC osteoblastic differentiation by preventing osteonectin up-regulation and although alpha-actin down-regulation is not affected, the molecular structure of the protein is typical of the functional one.
- A better understanding of the role of ECM is essential for the development of new treatments to counteract vascular calcification in uremia.

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