IRON CITRATE INHIBITS PHOSPHATE-INDUCED EARLY APOPTOSIS BY PREVENTING PHOSPHATIDYLSERINE TRANSLOCATION AND MITOCHONDRIAL DEPOLARIZATION

Mario Cozzolino[1], Paola Ciceri[1], Francesca Elli[1], Stefania Cannizzo[2] , Alessandra Mingione[1], Francesca Pivari[1], Antonella D'Arminio[2] and Giulia Marchetti[2]

[1]Laboratory of Experimental Nephrology and [2]Clinic of Infectious Diseases, Department of Health Sciences, University of Milan, Italy.

Background and Aims

• 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.

•We recently demonstrated that iron citrate strongly inhibits calcification by preventing nuclei DNA fragmentation and preserving the GAS6-Axl pro-survaival pathway.

•The aim of this study was to try to elucidate the effect of iron citrate on two early apoptotic markers such as phosphatidylserine translocation and mitochondrial membrane potential modification.

Materials & Methods

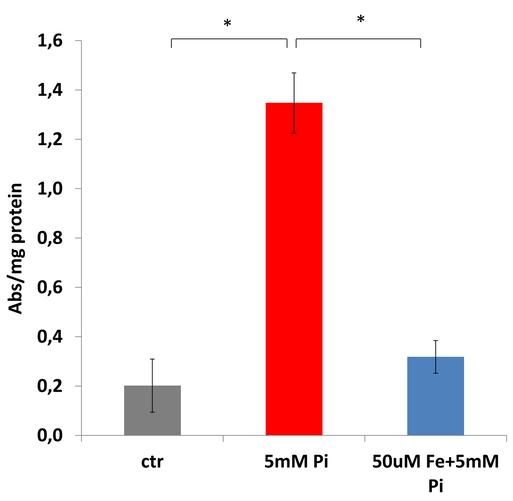
• Rat VSMCs were cultured and after 48h starvation they were challenged with inorganic phosphate (Pi) to induce calcification for 5 days. (Calcification medium: DMEM high glucose, 12% FBS, 10 mM sodium pyruvate, 100 U ml^{-1} penicillin and 0.1 mg ml^{-1} streptomycin and 50 ug/ml AA). Medium was replaced every 2 or 3 days.

•Flow cytometry was applied to determine the percentage of Annexin V positive/7-AAD negative cells and measure apoptosis. An Annexin V apoptosis detection kit with 7-AAD (BD, Palo Alto California) was used to measure the percentage of apoptotic cells.

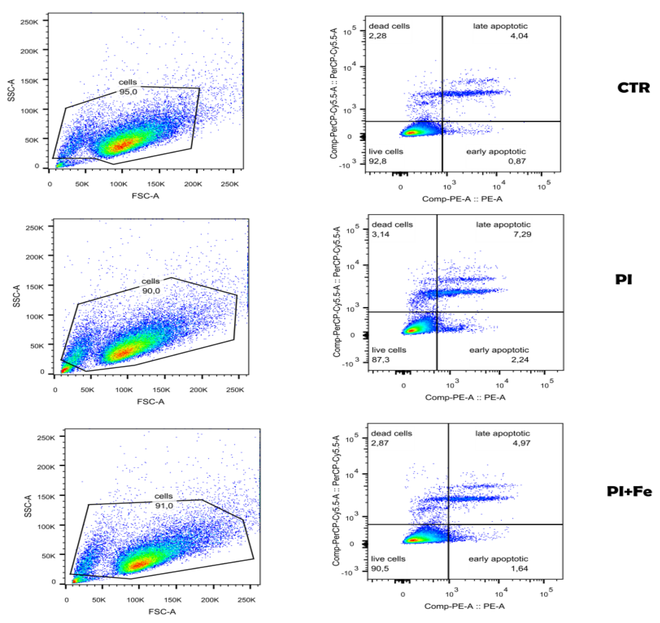
• 1 × 10^6 cells were resuspended in DMEM supplemented with 12% FBS and stained with 2.5 µg/mL JC-1. An incubation of 15 min at 37°C was followed. Cells were then washed and the pellet was resuspended in PBS for flow cytometric analysis. Emission of JC-1 monomers was detected in FL1 using a 520/30 nm bandpass filter; that of JC-1 aggregates in FL2 using a 590/30 nm bandpass filter. Mitochondrial depolarization was calculated as FL-2/FL-1 ratio.

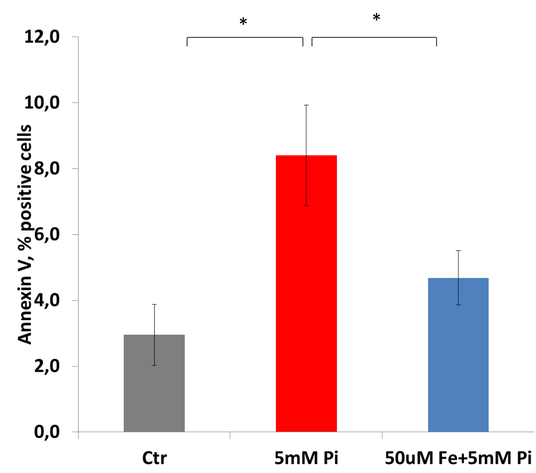
•All samples were analyzed using FACS-Verse BD cytofluorimeter.

Ferric Citrate Prevents High-Pi Induced Calcium Deposition

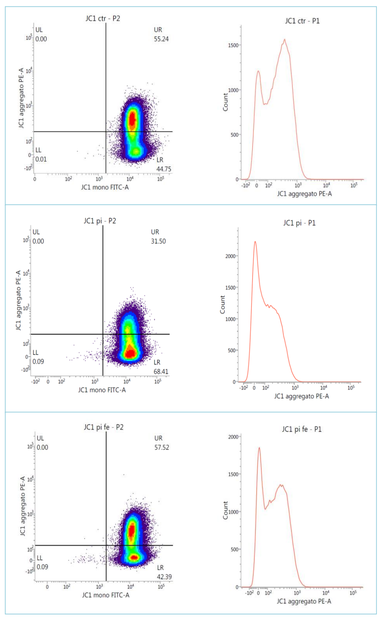


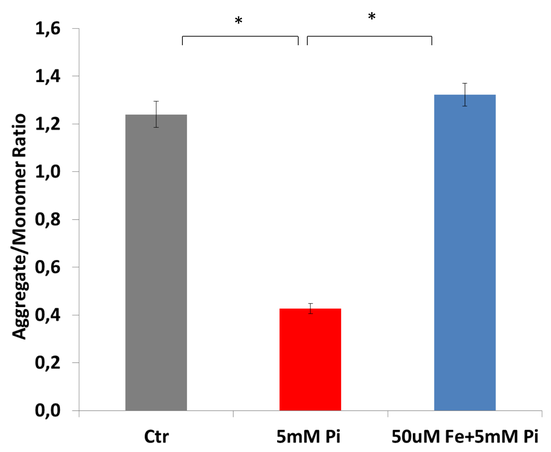
Ferric Citrate Prevents High-Pi Induced Early Apoptosis by Preventing Phosphatidylserine Translocation and Annexin V Binding





Ferric Citrate Prevents High-Pi Induced Early Apoptosis by Preventing Mitochondrial Depolarization





Conclusions

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

•Iron deeply affect Pi-induced apoptosis in the early phases almost completely abolishing Pi pro-apoptotic effect by preventing both phosphatidylserine translocation and mitochondrial membrane depolarization.

•Data obtained in our laboratory showed that Pi considerably alter mitochondria physiology after a prolonged chronic Pi-treatment inducing mitochondria calcification.

•Our data here demonstrate that the Pi-induced mitochondrion calcification is probably due, in the early stages, to modification of the membrane potential that is deeply involved in apoptosis.

•In conclusion, these in vitro data further support a beneficial role of iron administration in preventing Pi induced calcification by preventing apoptosis.

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