PHOSPHATE: A NOVEL RISK FACTOR FOR CARDIOVASCULAR DISEASE AND CKD PROGRESSION

SO051 DELETERIOUS EFFECTS OF PHOSPHATE ON VASCULAR FUNCTION

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Introduction and Aims: Elevated serum phosphate is an independent risk factor for cardiovascular disease. Whether this is a direct effect of elevated phosphate or dependent on changes in intracellular calcium or calcium/phosphate product is unknown. We examined the direct effects of phosphate concentration in human resistance vessels and human umbilical vein endothelial cells (HUVECs).

Methods: Surplus adipose tissue was removed from patients with chronic kidney disease (CKD) stage 5 undergoing live donor transplantation and their normal donors. Resistance vessels were dissected and incubated in a physiological saline solution (PSS) with normal (1.18 mM) or high phosphate concentration (2.5 mM) for 16 hours, then mounted on a myograph. Vasoconstrictor responses to phenylephrine (PE) and vasorelaxation responses to carbachol and sodium nitroprusside (SNP) were measured. Concentration-response curves were constructed for PE, carbachol and SNP. Area under the curve (AUC) was calculated and comparisons were made using either a t-test or an ANOVA. HUVECs were grown in normal (0.5 mM) and high (3 mM) phosphate concentration for 16 hours, then Gene expression was studied with PCR.

Results: Vessels from patients with and without CKD incubated in high phosphate relax less well to carbachol (p<0.05). Vessels from patients without CKD relax less well to SNP (p<0.05); this difference is not seen in vessels from patients with CKD.

Conclusions: Elevated phosphate decreases endothelium dependent vasodilatation in patients with and without CKD. This may be a marker of endothelial dysfunction, supported by the reduced eNOS protein expression and increased nitrotyrosine expression seen in HUVECs. Elevated phosphate also impairs endothelial independent relaxation in vessels from healthy patients without CKD. In vessels from healthy patients, elevated phosphate may alter cyclic GMP production and guanylate cyclase expression. These experiments indicate direct effects of elevated phosphate on the NO system, and on vascular function, and support the notion that phosphate has direct effects in uremia.

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SO052 MAGNESIUM SUPPLEMENTATION PREVENTS PHOSPHATE-INDUCED CALCIFICATION IN HUMAN AORTIC VASCULAR SMOOTH MUSCLE CELLS

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Introduction and Aims: Patients with chronic kidney disease (CKD) have a high prevalence of vascular calcification as a result of elevated blood phosphate (P). Consequently, cardiovascular disease is the leading cause of death in this population. The pathogenesis of vascular calcification is not well understood, but it appears to be a cell-mediated, dynamic and actively regulated process that resembles bone formation. Increasing evidence from in vitro, animal and clinical studies point to an inhibitory effect of magnesium (Mg++) on this calcification process. The aim of the present study was to delineate the molecular mechanism responsible for the inhibitory effect of Mg++-supplementation on P-sustained calcification in human aortic vascular smooth muscle cells.

Methods: Human aortic vascular smooth muscle cells (HaSMC) were cultured in DMEM containing 5% v/v fetal calf serum supplemented with MgCl2 and/or H2PO4 reaching a final concentration of 2 and 3 mM, respectively. HaSMC were harvested after 3 and 14 days and calcium (Ca++) and P deposition was determined. Additionally, total RNA was extracted at day 14 using Triol and used for Real-Time PCR analysis to screen for changes in mRNA expression of genes involved in calcification (RUNX2) and Mg++ homeostasis (TRPM7 and MagT1).

Results: Treatment of HaSMC using H2PO4 resulted in Ca++ (Fig 1a) and P depositions (not shown) after 14 days, which were prevented by Mg++-supplementation. Additionally, upregulation of RUNX2 a marker of calcification in high-phosphate conditions was prevented by Mg++-supplementation whereas the expression levels of TRPM7 and MagT1, two genes involved in Mg++ homeostasis, remained unaltered (Fig 1b).

Conclusions: Our results demonstrate the potential of Mg++-supplementation in the prevention of HaSMC calcification that warrants further investigation of genes involved in Mg++-homeostasis (e.g. TRPM6 and CNNM2).