Long-term Treatment with Montmorillonite-Illite Clay Mineral Reduces Uremic Toxins and Vascular Pathologies in Rats with Chronic Renal Failure

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INTRODUCTION

Chronic kidney disease (CKD) is highly associated with elevated serum phosphate levels contributing to the increased cardiovascular risk observed in dialysis patients. Hyperphosphatemia initiates pathological cascades such as the phenotypic switch in vascular smooth muscle cells from a contractile to a synthesizing character, hypertrophy and hypertension. This in turn leads to an accelerated progressive vascular calcification which is strongly associated with future cardiovascular morbidity and mortality. Hypercreatininemia and microalbuminuria are associated with cardiovascular risk as well. Both are reliable markers for cardiovascular disease, stroke and myocardial infarction. Despite a low phosphate diet, phosphate accumulates progressively and even dialysis fails to adequately prevent hyperphosphatemia. A low phosphate diet, an efficient removal of phosphate load by dialysis and the administration of phosphate binders are the premise to keep hyperphosphatemia under control. Similar to clinical adsorbers, Montmorillonite-Illite clay minerals possess a great phosphate affinity. Its specific four-layer structure, the good ion exchange capacity and high levels of iron oxyde qualify Montmorillonite-Illite clay minerals as potent phosphate adsorber which has little side effects (Fig. 1).

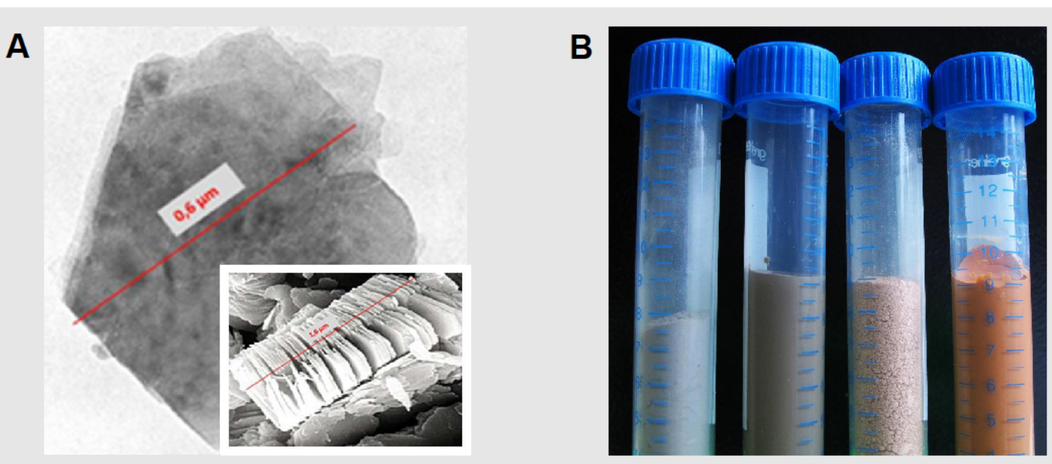

***Fig. 1**: (A) Electron micrographs of Montmorillonite-Illite Silikats (FIM BIOTECH). (B) Processed clay minerals before and after colloidal grinding and calcination methods (from left to right).*

AIMS AND OBJECTIVES

The aim of this study was to investigate the phosphate adsorbtion capacity of Montmorillonite-Illite clay mineral in comparison to the clinically used phosphate binder Fosrenol. The effect on the intracellular calcium balance of arterial smooth muscle cells *in vitro* as well as the *in vivo* impact on (i) kidney retention parameters, (ii) microalbuminuria and (iii) pathological vascular changes should be determined in rats with 5/6 nephrectomy-induced CKD.

MATERIALS AND METHODS

In vitro phosphate binding capacity was analysed by measuring the cellular calcium concentration and detection of calcium depositions in human coronary artery smooth muscle cells (HCASMC) after incubation with high phosphorus cell culture medium in the presence or absence of either Montmorillonite-Illite clay mineral (FI5ppVn) or Fosrenol. *In vivo*, CKD rats induced by 5/6 nephrectomy received a high phosphate and calcium diet supplemented with either Montmorillonite-Illite clay mineral or Fosrenol. Both, urinary and serum levels of uremic toxins were determined in the course of CKD pathogenesis. Pathological arterial changes were determined by evaluation of histological aortic sections.

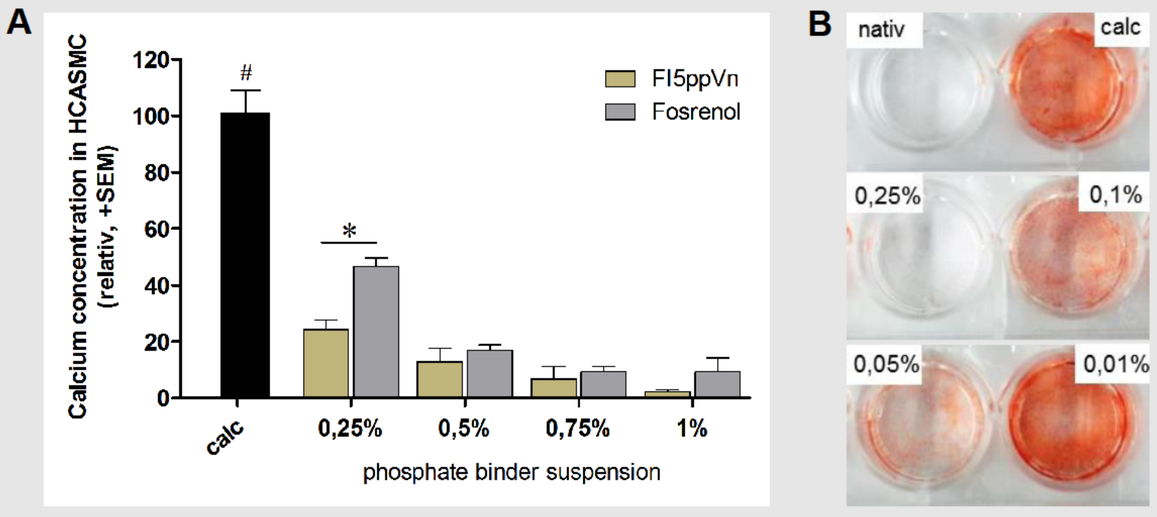

***Fig. 2**: Reduction of intracellular calcium concentration (A) and calcium deposits detected via Alizarin red staining in HCASMC after incubation with high phosphorus medium in the presence or absence of Montmorillonite-Illite clay mineral and Fosrenol (+SEM, p≤ 0,05; #p ≤ 0,05 calcified HCASMC versus adsorber treated, calcified HCASMC).*

RESULTS

β-glycerophosphate added culture medium induced cellular calcification in HCASMC detectable e.g. via Alizarin red staining and increased intracellular calcium concentration when incubated with calcification medium, whereas Montmorillonite-Illite clay mineral treatment significantly reduced calcium concentrations and its depositions similar to Fosrenol treated cells (Fig. 2).

Treatment of 5/6 nephrectomized (NX) rats with Montmorillonite-Illite clay mineral significantly decreased kidney retention parameters such as serum phosphate and creatinine as well as urinary microalbumin levels (Fig. 3). Severe vascular pathology was observed in 5/6NX rats by arterial wall thickness analyses, extracellular matrix (ECM) and αSMA staining. Due to CKD in combination with a high phosphate diet arterial walls were significantly thickened. Treatment with Montmorillonite-Illite clay mineral prevented this hypertrophy (Fig. 4). Furthermore, ECM levels were significantly reduced in 5/6NX rats that received mineral treatment, comparable to sham-operated littermates (Fig. 5). As a result of hyper-phosphatemia, vascular cells lost its contractile character due to reduced αSMA and increasingly develop a synthesizing character. Mineral treatment prevented the reduction of αSMA levels in CKD rats and preserve its ability to contract (Fig. 6).

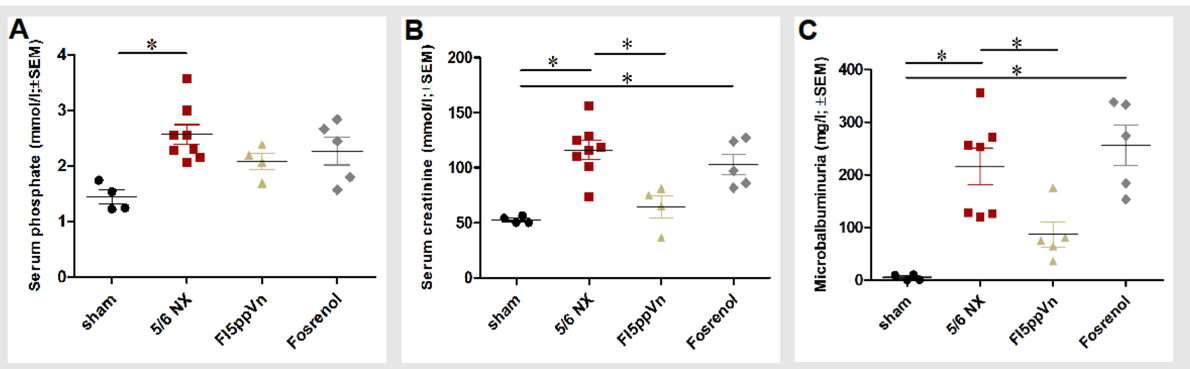

***Fig. 3**: Montmorillonite-Illite clay mineral treatment significantly decreased a CKD related hyperphosphatemia and hypercreatininemia and reduced microalbuminuria induced by 5/6 nephrectomy in rats (+SEM, p≤ 0,05).*

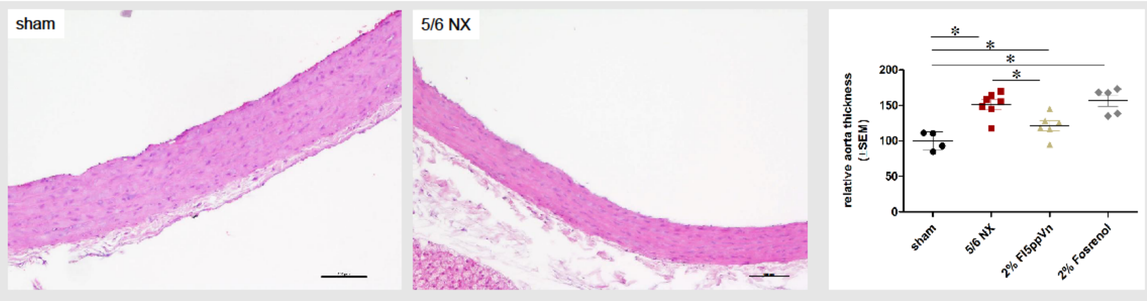

***Fig. 4**: Treatment with clay mineral prevented the thickening of the vascular walls of the abdominal aorta caused by high phosphate diet in 5/6 nephrectomized rats (+SEM, p≤ 0,05).*

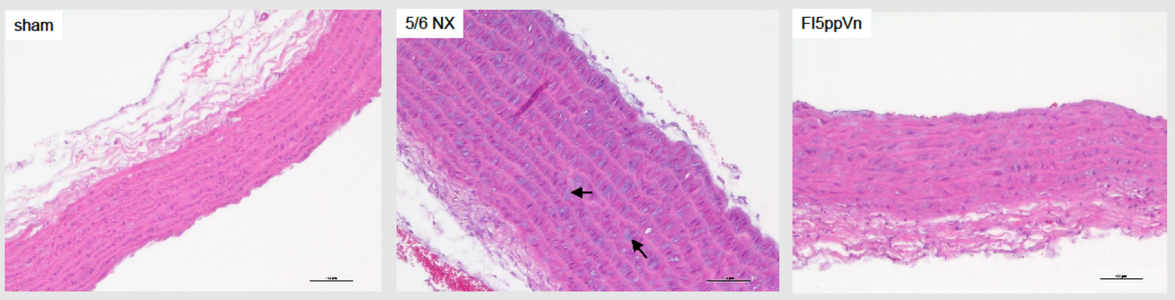

***Fig. 5**: Montmorillonite-Illite clay mineral treatment reduced the synthesis of ECM within the tunica media (black arrows) and prevented the expansion of the tunica intima in consequence of hyperphosphatemia (+SEM, p≤ 0,05).*

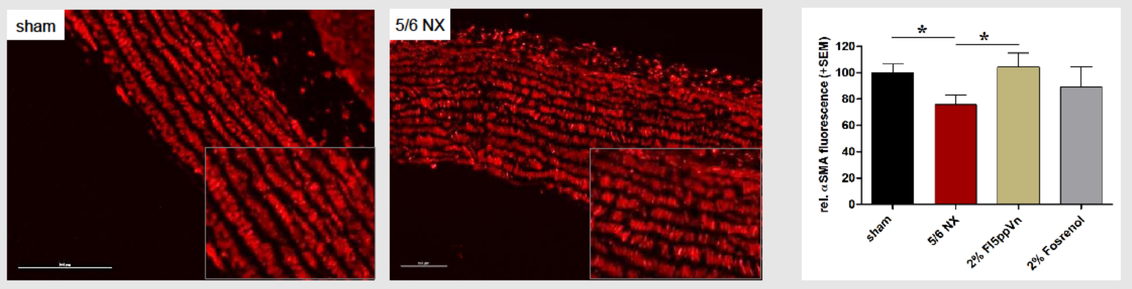

***Fig. 6**: αSMA immunofluorescence staining of aortic arches of 5/6 nephrectomized rats implicates a reduction of smooth muscle actin in consequence of vascular changes caused by CKD was prevented by Montmorillonite-Illite clay mineral treatment detected by quantification analyses of fluorescence intensities (+SEM, p≤ 0,05).*

CONCLUSION

The present study indicates Montmorillonite-Illite clay mineral as highly potent phosphate absorber both *in vitro* and *in vivo*, compared to an established clinical absorber. Moreover, our findings of reduced hypertrophy and microalbumin excretion after Montmorillonite-Illite clay mineral diet is of particular interest for patients as well as clinicians since the presence of hypercreatininemia and microalbuminuria has been associated with an increased risk for cardiovascular disease.

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J6) Chronic Kidney Disease. Nutrition, inflammation and oxidative stress.
Jacqueline Hofrichter
DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.
52nd ERA-EDTA CONGRESS London 2015 UNITED KINGDOM MAY 28TH
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