Duality of calcitriol-based inhibition or propogation of vascular calcification *in vitro* is dependent on normal or high calcium levels, respectively.

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Introduction

Background: Secondary hyperparathyroidism (SHPT) is a hallmark of chronic kidney disease (CKD) resultant of reduced calcitriol (1,25-dihydroxyvitamin D_3) production. To ameliorate SHPT, calcitriol supplementation is a common therapy for CKD patients. This calcitriol supplementation has been found to both increase and decrease the occurrence of vascular calcification (VC), a risk factor of cardiovascular disease. Where *in vitro* studies on vascular smooth muscle show increases in VC, *in vivo* studies in rat models of CKD display both reduction and propagation of VC. Further, clinical studies associate calcitriol supplementation with improvements in CKD patient survival. The mechanisms by which calcitriol mediates the beneficial and adverse effects on the vasculature are not established. One potential avenue is through altering the expression of the extracellular matrix remodeling protein, Matrix Metalloproteinase 2 (MMP-2).

Purpose: The study's aim was to identify potential mechanisms for the duality of calcitriol's effects on the vasculature in CKD; specifically, to examine mineral accrual and alterations to remodeling proteins that support VC.

Methods

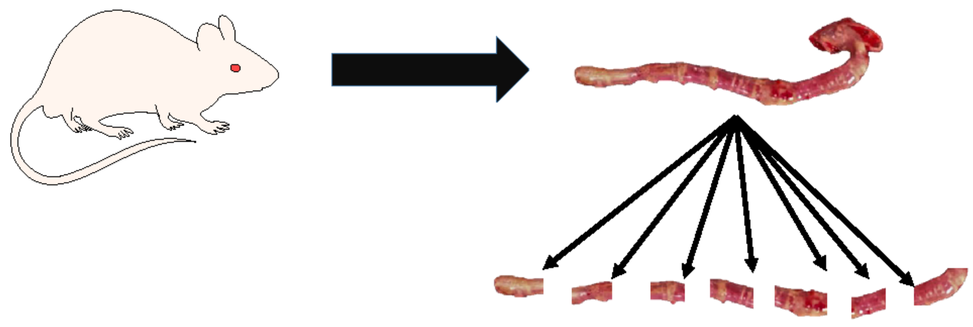

Figure 1. *In vitro* model of CKD for arterial segments. Aortic tissue was harvested from 16 week-old Sprague Dawley rats (n = 12), with whole rings incubated in Dulbecco's Modified Eagles Medium (DMEM) for 4 days. Incubation treatments consisted of control (Group 1, 0.9mM PO_4^{3-} & 1.6mM Ca^{2+}), high Ca^{2+}/low PO_4^{3-} (Group 2, 0.9mM PO_4^{3-} & 3.2mM Ca^{2+}), normal Ca^{2+}/high PO_4^{3-} (Group 3, 3.8mM PO_4^{3-} & 1.6mM Ca^{2+}), and high Ca^{2+}/high PO_4^{3-} (Group 4, 3.8mM PO_4^{3-} & 1.6mM Ca^{2+}). Calcitriol (0nM, 1nM, 10nM, or 100nM) was added to different DMEM treatments. Media was switched every 48 hours.

Results

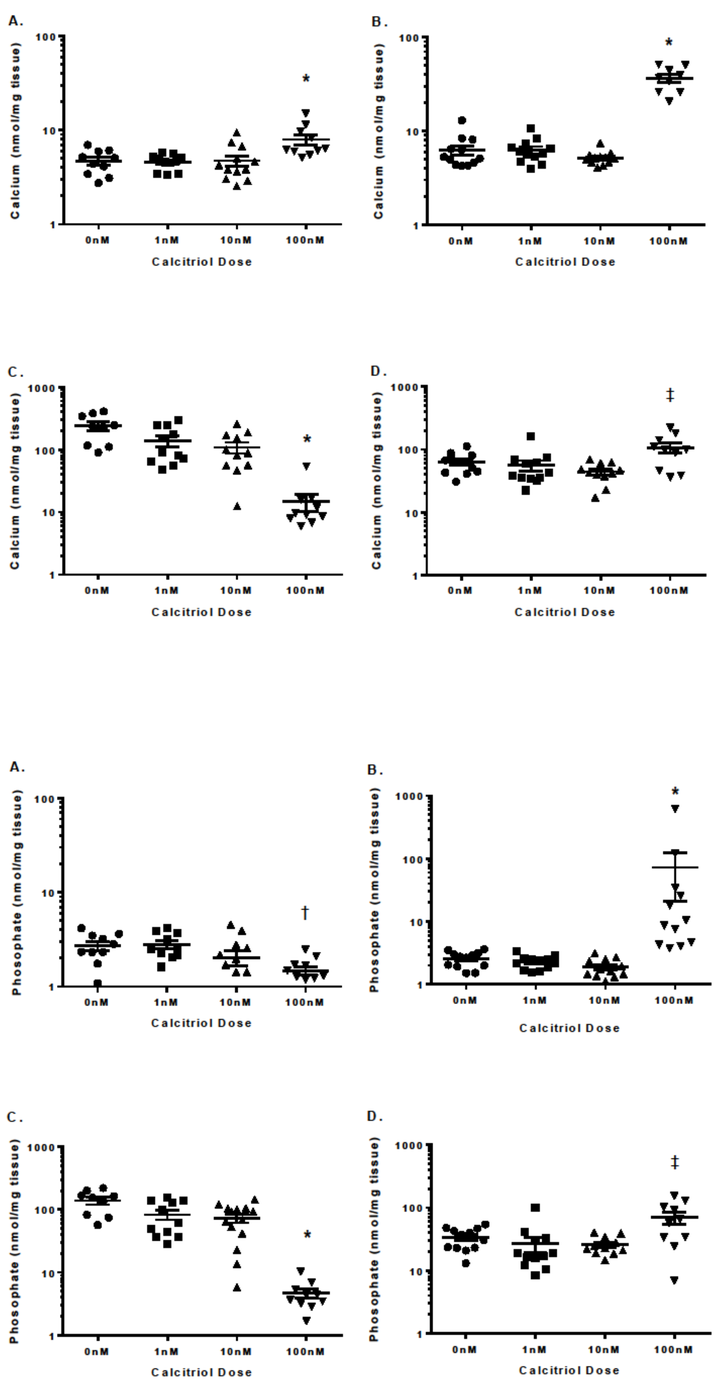

Figure 2. Calcitriol's effect on calcium accrual: Dependency on both media calcium and phosphate concentrations. Calcium accrual in aortic rings across treatments. Incubated in (A) 0.9mM PO_4^{3-} with 1.6mM Ca^{2+}, (B) 0.9mM PO_4^{3-} with 3.2mM Ca^{2+}, (C) 3.8mM PO_4^{3-} and 1.6mM Ca^{2+}, and (D) 3.8mM PO_4^{3-} and 3.2mM Ca^{2+}. * Significantly different than all groups ($p<0.05$). ‡ Significantly different than 1nM and 10nM ($p<0.05$). Data expressed as mean ± SEM.

Figure 3. Calcitriol's effect on phosphate accrual: Dependency on both media calcium and phosphate concentrations. Phosphate accrual in aortic rings across treatments. Incubated in (A) 0.9mM PO_4^{3-} with 1.6mM Ca^{2+}, (B) 0.9mM PO_4^{3-} with 3.2mM Ca^{2+}, (C) 3.8mM PO_4^{3-} and 1.6mM Ca^{2+}, and (D) 3.8mM PO_4^{3-} and 3.2mM Ca^{2+}. +. * Significantly different than all groups ($p<0.05$). † Significantly different than 0nM and 1nM ($p<0.05$). ‡ Significantly different than 1nM and 10nM ($p<0.05$). Data expressed as mean ± SEM.

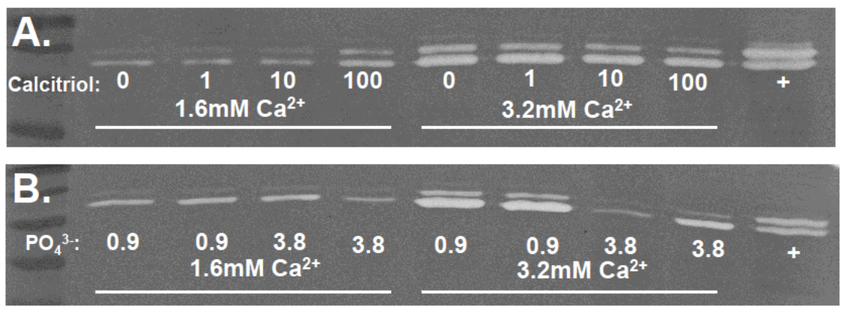

Figure 4. Calcium mediated increases in MMP-2 activity and expression are independent, in part, of calcitriol. Zymogram representation of MMP-2 expression and activity. (A) Calcitriol dose effect in 0.9mM PO_4^{3-} and 1.6mM Ca^{2+} vs 3.2mM Ca^{2+} conditions. (B) Comparison of the effects of calcium exposure (1.6mM vs 3.2mM) on MMP-2 activity in 0.9mM PO_4^{3-} and 1nM Calcitriol exposure.

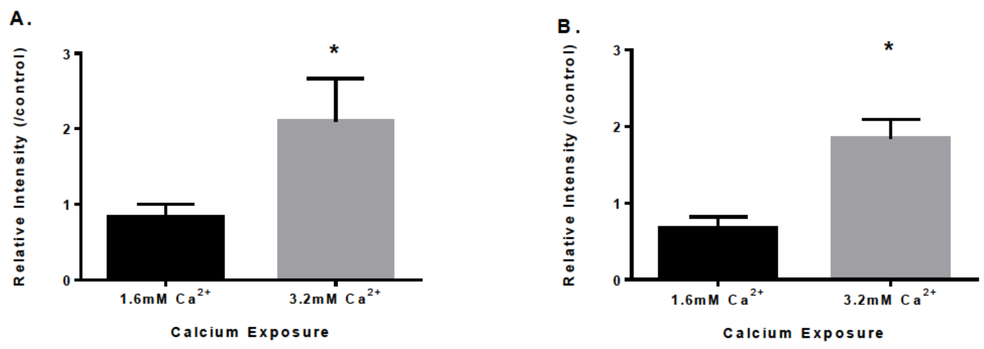

Figure 5. High calcium concentration increases total MMP-2. Total MMP-2 (relative to standard) in media with 0.9mM PO_4^{3-} and (A) 0nM Calcitriol, or (B) 100nM Calcitriol.* Significantly different ($p<0.05$).

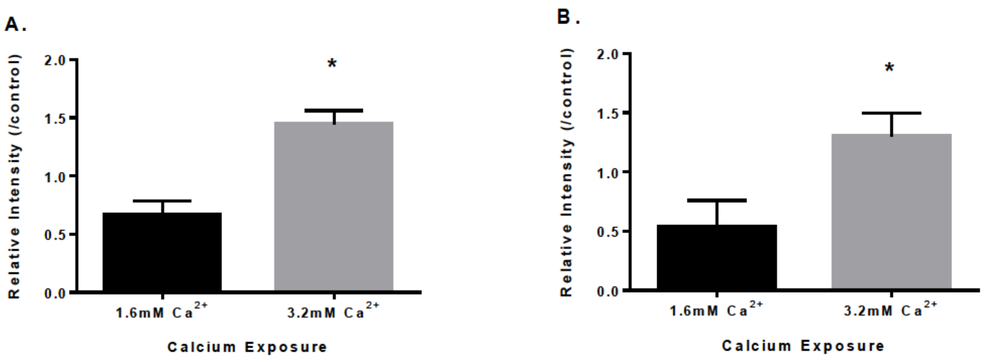

Figure 6. High calcium concentration increases active MMP-2. Active MMP-2 (relative to standard) in media with 0.9mM PO_4^{3-} and (A) 0nM Calcitriol, or (B) 100nM Calcitriol. *Significantly different ($p<0.05$).

Summary and Conclusions

1. Calcitriol reduces VC in a dose-dependent manner in a hyperphosphatemic – normal calcium environment; and yet, calcitriol increases calcium accumulation in a normal phosphate environment
2. Hyperphosphatemia and high calcium abolish calcitriol-based reductions to VC
3. Calcium increases active and total expression of MMP-2

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