The role of secondary calciprotein particles in the mineralisation paradox of chronic kidney disease

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Background

In health, tightly controlled processes restrict mineralisation to bone and teeth. The process is disturbed in chronic kidney disease (CKD), where there is a parallel, but paradoxical, development of vascular calcification, and reduction in bone mineralisation (Figure 1), resulting in cardiovascular mortality and increased fracture incidence.

Fig 1a: Increased cardiovascular mortality in ESKD. (ANZDATA registry[1])

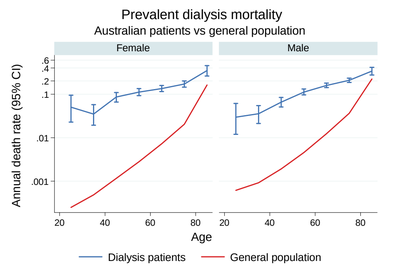
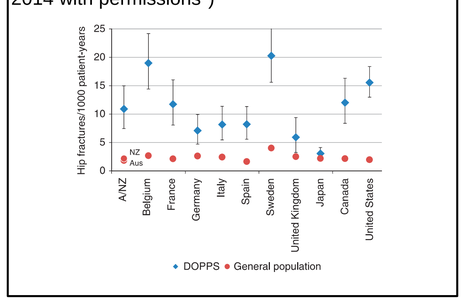


Fig 1b: Fracture rates are higher in the dialysis population (Blue dots). (Tentori *et al*, *Kidney Int* 2014 with permissions[2])



The mechanism behind this phenomenon is not clear, but circulating nanometer-sized calciprotein particles (CPP) exist in CKD patient serum and may be relevant. These contain a calcium phosphate mineral core with a fetuin-A rich protein shell[3]. Serum CPP levels have been associated with arterial stiffness, arterial calcification and increased mortality in CKD patients[3,4]. It is unknown whether CPP affects bone and VSMC mineralisation to explain these clinical observations.

Aim

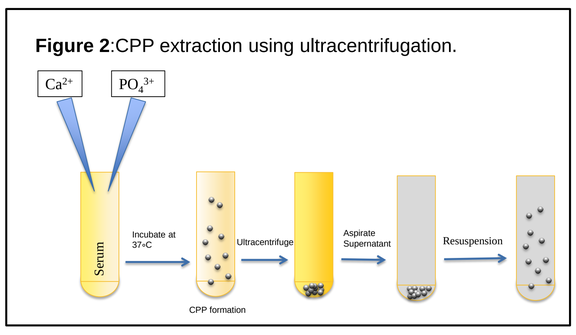
To investigate the effect of secondary CPP on mineralisation of osteoblasts and vascular smooth muscle cells (VSMC) in culture.

Methods

CPPs were synthesized by mixing serum with buffered calcium (Ca) and phosphate (Pi) solutions (Fig. 2)[5]. All CPP used in experiments were secondary CPPs containing crystalline calcium phosphate mineral phases. The concentration is expressed as the amount of CPP bound calcium in media.

Saos-2 (an osteosarcoma cell line) and MOVAS (murine VSMC) were treated for 7 days in control media (ConM, 1mM Pi), osteogenic media (PiM, 4mM Pi), CPP2 supplemented media (CPP2M) and Pi and CPP2 supplemented media (Pi+CPP2M). In some experiments, Pi+CPP2M were preincubated at 37◦C for 24 hours before putting onto the cell monolayer.

Figure 2:CPP extraction using ultracentrifugation.

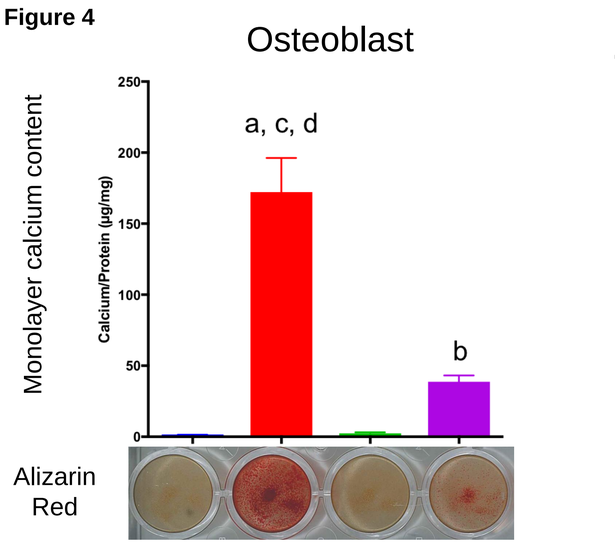


Cell monolayer mineralisation was quantified by its total monolayer calcium or visualised using Alizarin Red staining. Cell viability was determined using tryphan blue exclusion method. ALP activity was assayed using the p-nitrophenyl phosphate method. Intracellular calcium was determined using a flow cytometer after Fluo-4 staining.

Results

In high Pi media, CPP2 reduced mineralisation in osteoblasts, but paradoxically increased mineralisation in VSMC (Figure 4).

Figure 4



	ConM	PiM	CPP2M	Pi+CPP2M
Pi (3mM)	-	+	-	+
CPP2 (µg/ml)	0	0	10	10

	ConM	PiM	CPP2M	Pi+CPP2M
Pi (3mM)	-	+	-	+
CPP2 (µg/ml)	0	0	10	10

Legend: a, P<0.05 vs ConM; b, P<0.05 vs PiM; c, P<0.05 vs CPP2M, d, P<0.05 vs Pi+CPP2M

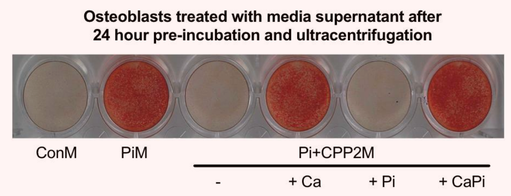
Mechanisms of reduced osteoblast mineralisation

1. CPP2 reduces bioavailable calcium

CPP2 contains hydroxyapatite crystals, a mineral phase with low solubility. These crystals can promoting further Ca and Pi ion accretion. To examine the hypothesis that CPP2 accrue Ca Pi, media was pre-incubated by 24 hours and then Ca and Pi measured in the supernatant after ultracentrifugation.

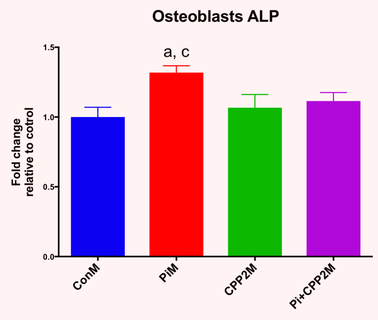
Pi+CPP2M supernatant contains less Ca and Pi than that of PiM. Figure 5 showed that replacement of Ca (+Ca), not Pi (+Pi) restored osteoblast mineralisation in the Pi+CPP2M supernatant.

Figure 5



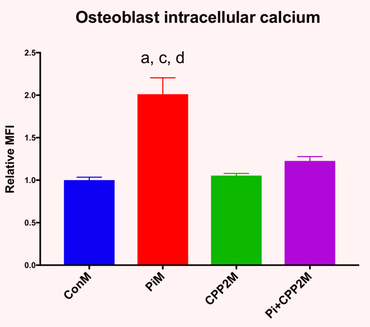
2. CPP2 attenuated osteoblast ALP activity.

Figure 6



3. CPP2 reduced $[Ca^i]$

Figure 7

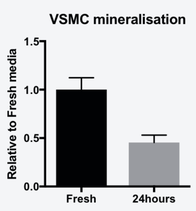


Mechanisms of increased VSMC mineralisation

1. Passive deposition of CPP2 does not entirely explain VSMC mineralisation.

After 24 hours of incubation, CPP2 contains more Ca and Pi, thereby making the particles larger and more prone to sedimentation. VSMC treated with 24 hours incubated Pi+CPP2M had less mineralisation compared to fresh media (Figure 9).

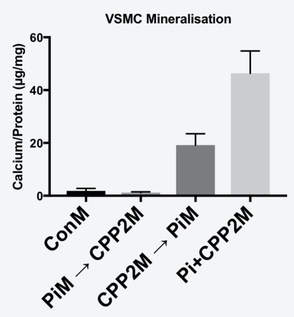
Figure 9



2. CPP2 seeded VSMC mineralisation in high Pi media.

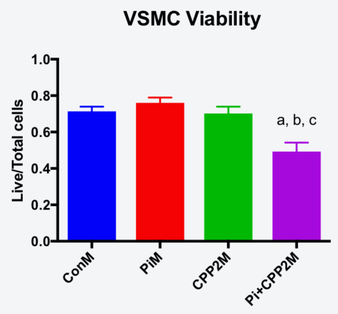
VSMC were treated with either: ConM for 7 days; PiM → CPP2M, PiM media for the first 3.5 days followed by CPP2M media for 3.5 days; CPP2 → Pi, CPP2M for the first 3.5 days followed by PiM for 3.5 days; or Pi+CPP2M for 7 days as the positive control (Figure 10).

Figure 10



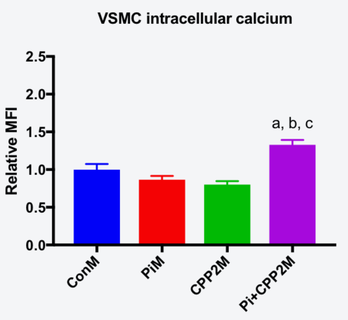
3. CPP2 and Pi reduced VSMC viability.

Figure 11



4. CPP2 and Pi increased VSMC $[Ca_i]$ on Day 7

Figure 12



Conclusion

1. **CKD milieu is characterised by hyperphosphataemia and the presence of circulating CPP2. In an *in vitro*, high phosphate environment, crystalline mineral containing CPP reduced osteoblast mineralisation, but paradoxically increased VSMC mineralisation.**
2. **Pi and CPP2 reduced bioavailable media Ca, which consequently inhibited osteoblast mineralisation. Pi and CPP2 also attenuated important osteoblastic activities such as ALP and intracellular calcium accumulation.**
3. **In VSMC, CPP2 seeded mineralisation and high Pi propagated further mineralisation. The effects on reduced cell viability and increased intracellular calcium warrants further mechanistic studies.**

References

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