Editorial Comments

What dishwashers and humans have in common

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One answer to the title question could be that both ‘species’, dishwashers and humans, can be protected from unwanted calcification by the anticalcifying properties of small double-phosphate molecules, diphosphonates or pyrophosphates, respectively. More than 40 years ago, we started to realize that there are compounds present in many biological fluids capable of inhibiting precipitation of calcium phosphates [1]. These compounds have been identified as inorganic pyrophosphates (P–O–P) and have been demonstrated to slow the transformation of amorphous calcium phosphate into its crystalline form while inhibiting crystal aggregation. This observation led to the development of diphosphonates, in which the P–O–P bond is replaced by a P–C–P bond and, subsequently, to the development of bone scanning agents and bisphosphonates.

While excellent evidence has since accrued that diphosphonate detergents protect dishwashers from premature calcification and ‘sudden death’—and, thus, contribute to longevity—this evidence is still weak and just starting to accumulate in mammals and human beings. The paper by O’Neill, Sigrist and McIntyre in this issue of Nephrology Dialysis Transplantation is one step forward in exploring the potential clinical role of endogenous pyrophosphates in human disease scenarios and, consequently, addresses the patient population at highest risk for unwanted calcification, i.e. individuals suffering from advanced chronic kidney disease (CKD) [2]. The authors measured plasma pyrophosphate concentrations in 115 individuals in CKD stages 4–5D and correlated these levels with the degree and change in large artery calcification which was quantitatively detected at the superficial femoral artery by computed tomography (CT). CT imaging was obtained at baseline and after 1 year. An inverse association was found between baseline calcification score and pyrophosphate levels; also, an inverse, though non-significant trend regarding calcification progression and pyrophosphate levels was reported. In the past and over many years, this group of investigators has led the path towards this current and significant observation with both key experimental and preliminary clinical contributions [3–7].

Pyrophosphate synthesis can proceed ubiquitously all over the body [8]. Tissue pyrophosphate release is regulated by the following three factors (Figure 1): first, the rate-limiting enzyme ‘ecto-nucleotide pyrophosphatase phospho-diesterase-1’ (ENPP-1); second, the transmembrane transporter ‘ANK’ encoded by the ‘progressive ankylosis’ locus; and third, the membrane-bound enzyme ‘tissue non-specific alkaline phosphatase’ (TNAP). Activity of ENPP-1 determines the production rate of pyrophosphates, and intact ANK secures extracellular availability by specifically transporting pyrophosphates out of the cells. However, TNAP degrades secreted pyrophosphates into single phosphate ions and, thus, an increased enzyme activity would cause local hyperphosphataemia. Both genetic ANK and ENPP-1 deficiencies in mice cause phenotypes of dystrophic calcification [9,10]. In humans, a loss-of-function mutation of ENPP-1 has been identified as causing a disastrous disorder termed idiopathic infantile arterial calcification (IIAC) leading to severe vascular calcifications in newborns and small children [11].

This paper by O’Neill et al. is of significant relevance because observations like these may pave the way to novel therapeutic pathways and strategies, but numerous questions remain unanswered. Do plasma pyrophosphate concentrations reliably represent tissue levels and the production status of pyrophosphates? How are they influenced by sequestration, degradation or removal (dialysis)? With regard to sequestration, could it be possible that the state of bone mineralisation (trapping of pyrophosphates) overrules all other determinants influencing plasma concentrations? How does primary endothelial or vascular damage impact on plasma pyrophosphate levels? Taken together, will pyrophosphate measurements have any potential at all to become a relevant risk biomarker for CKD patients in the future? At the moment, there will be no definite answer to any of these questions, but the strength of this paper is that it opens a pathophysiological concept for future clinical exploration enabling appropriate answers.

Speculating about novel strategies of calcification risk evaluation, it may become an attractive approach to look into composite patterns of calcification inhibitors. We are progressively gaining additional insights into the roles of systemic glycoproteins (fetuin-A and calciprotein particles), vitamin K status (matrix Gla protein carboxylation)
and the osteoprotegerin/RANK-ligand system in calcification protection. Consequently, future researchers may want to elaborate on the question of which combination of inhibitor deficiencies may be the most worrisome in CKD [8].

What potential treatment options may arise from the observation that pyrophosphate deficiency may be a key risk factor of vascular calcification in CKD, or even in the general population, and can a potential clinical role for pyrophosphates thus be confirmed in the future? The following three mechanistic options are imaginable:

- stimulation of synthesis = induction of ENPP-1;
- prevention of degradation = inhibition of TNAP; or
- pyrophosphate replacement = bisphosphate therapy.

Concerning the first option, we are currently not aware of any feasible strategy to specifically induce ENPP-1, so this may remain wishful thinking for the time being. There are, however, inhibitors of alkaline phosphatase (AP) activity [4], but since this enzyme is so abundantly expressed throughout the body, it may become excessively difficult to design an inhibitory compound specifically capable of distinguishing the ‘good’ from the ‘bad’ AP isoforms.

Concerning the third approach, bisphosphonates, we have data. Experimentally, there are numerous reports on bisphosphonates as potent anticalcifying agents in animal models and as inducers of small protein–mineral complexes which potentially serve as intermediate clearance molecules for small preformed hydroxyapatite crystals [5, 12–14].

Decades ago, diphosphonates were successfully used to treat myositis ossificans [15]. From our point of view, the most convincing information, however, comes from a small case series of newborn children suffering from IIAC [16]. Treatment of two children with etidronate immediately after birth probably saved their lives by attenuating and dissolving severe vascular calcification; near-total aortic medial calcification disappeared within weeks after treatment initiation. There is also a case report that pamidronate improved calciphylaxis in a uraemic CKD patient, and Nitta and colleagues reported beneficial effects on coronary artery calcification progression in a cohort of dialysis patients by etidronate treatment [17,18]. In advanced CKD, there are, however, uncertainties concerning the half-life and fate of bisphosphonates and the risk of inducing or aggravating adynamic bone disease (ABD) by this group of compounds. ABD by itself is considered a risk factor for cardiovascular calcification and morbidity in CKD patients. Further, we do not know, whether pyrophosphate-like effects can systemically be induced by all bisphosphonates or potentially more effectively or even exclusively by the ‘prototypic’ compounds such as etidronate. So overall, the risk-benefit ratio of bisphosphonates in calcified CKD patient remains unclear, and such treatment may even be harmful in some individuals.

In conclusion, the high burden of cardiovascular calcification is still a ‘black box’; the processes are multifactorial, and one player is never enough. Relative or absolute pyrophosphate deficiency may, however, be a big player in this context and one that may ultimately become tackled effectively. In the case of dishwashers, it took a few years to develop effective diphosphonate detergents to keep ‘vessels’ clean and soft. In humans, we are just at the beginning.

Conflict of interest statement. None declared.


References

The endoplasmic reticulum (ER) provides a unique environment for appropriate protein folding and assembly to produce functional, mature proteins. A number of pathophysiological insults cause accumulation of unfolded proteins in the ER, namely, ER stress. In response to ER stress, cells adapt themselves to the stress conditions via the unfolded protein response (UPR), leading to attenuation of translation, induction of ER chaperones and activation of ER-associated degradation (ERAD) to eliminate immature proteins. The UPR is involved in a diverse range of pathophysiological events [1,2], including renal diseases [3].

In response to ER stress, three major branches of the UPR are activated, as summarized in Figure 1A. Those include the RNA-dependent protein kinase-like ER kinase (PERK) pathway, the activating transcription factor 6 (ATF6) pathway and the inositol-requiring ER-to-nucleus signal kinase 1 (IRE1) pathway. Activation of PERK causes phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α), which leads to general inhibition of protein synthesis. In response to ER stress, p90ATF6 transits to the Golgi where it is cleaved by the proteases SIP and S2P, yielding an active transcription factor p50ATF6. Similarly, activated IRE1 catalyses removal of a small intron from the mRNA of X-box binding protein 1 (XBP1). This splicing event creates translational frameshift in XBP1 mRNA to produce an active transcription factor. Active p50ATF6 and XBP1 subsequently bind to the ER stress response element (ERSE) and the UPR element (UPRE), leading to expression of target genes including ER chaperones and ERAD factors that degrade unfolded proteins.

Cyclosporine A (CsA) and tacrolimus (FK506) are pivotal immunosuppressive agents to prevent allograft rejection in renal transplantation. Use of these immunosuppressants leads to significant reduction in the incidence of acute graft rejection and improvement in the survival of kidney transplants [4]. CsA binds to the cyclophilin family of molecules that have high affinity for calcineurin, a key protein phosphatase in the activation of T cells. FK506 is another calcineurin inhibitor, the mechanism of which is similar to that of CsA. It forms complexes with its cytosolic partner FK506-binding protein 12, and the complexes bind to calcineurin. By blocking calcineurin, CsA and FK506 inhibit phosphatase-controlled translocation of nuclear factor of activated T-cells (NF-AT) into the nucleus and prevent induction of cytokines and their receptors required for activation and proliferation of lymphocytes and other immune cells [5]. These agents are, therefore, regarded as inhibitors of immune cell function. However, several reports have

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