Beyond phosphate—role of uraemic toxins in cardiovascular calcification

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Introduction

Vascular calcification (VC) is a hallmark of atherosclerosis and has been linked to increased cardiovascular disease and mortality. VC is highly prevalent in patients with chronic kidney disease (CKD), in whom it occurs more frequently and progresses more rapidly than in the general population [1–3]. In CKD patients, cardiovascular mortality increases exponentially with age and is up to 500 times higher than in the general population [4]. The presence of VC is independently predictive of subsequent cardiovascular disease and mortality, beyond established conventional risk factors [5,6]. VC develops at two sites of the arterial wall: the intima and the media. The survival of CKD patients with arterial media calcification is longer than in patients with arterial intima calcification, whose survival in turn is significantly shorter than that of VC-free patients [7].

Metabolic disturbances in CKD involving calcium, phosphorus, parathyroid hormone and vitamin D, and exogenous factors including excessive vitamin D and calcium intake contribute to the initiation and progression of VC. Disorders of mineral and bone metabolism in CKD patients (CKD-MBD) are associated with an increased risk for cardiovascular calcification, morbidity and mortality [8]. Among them, increased serum phosphorus and serum calcium–phosphorus ion product have been shown to correlate with progressive vascular and/or valvular calcification and mortality in numerous observational studies [2,9]. However, evidence has been accumulating that soft tissue calcification is not only a passive process involving calcium and phosphate precipitation due to low ion solubility in serum, but also an active process involving numerous players. The major role of circulating and local promoters and inhibitors of extraosseous calcification has been progressively recognized in recent years [10]. The complex interaction between active and passive processes guarantees the prevention of soft tissue calcium phosphate deposition under physiological circumstances. Disturbances of this subtle balance in CKD lead to calcification of blood vessels and other soft tissues. A better understanding of the underlying mechanisms is of great importance for the treatment and prevention of this dramatic complication. It may eventually allow us to improve the poor prognosis of these patients.

Lessons from experimental studies in vitro and in vivo

Vascular smooth muscle cells (VSMCs) maintained in culture can undergo phenotypic changes secondary to an increase in medium phosphate concentration, towards a phenotype of osteoblast-like cells [11]. Increased phosphate and calcium levels in the incubation milieu synergistically and independently induce VSMC phosphate uptake [12]. The relative importance of the two ions in this process remains a matter of debate [13]. Under high incubation medium calcium/phosphate conditions, the surrounding extracellular matrix undergoes mineralization along a process resembling that of bone. However, as aforementioned, high calcium and/or phosphorus concentrations are only part of the game in a complex, multistep process. A large number of factors are involved in the initiation of vascular calcification. Alkaline phosphatase (ALP) is one of the phenotypic markers of osteoblast-like activity within the VSMC layer. Calcifying, VSMC-derived cells express high levels of ALP, and their calcification activity is highly dependent on the degree of ALP activity [14,15], possibly through degradation of pyrophosphate which is physiologically

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protective [16]. A higher expression of osteocalcin, osteonectin and bone Gla protein is also associated with the intensity of the transformation of VSMC towards a calcifying phenotype [17,18]. In contrast, other local or circulating proteins such as osteopontin, matrix-Gla protein, fetuin-A, osteoprotegerin, FGF-23 and klotho function as potent inhibitors of ectopic calcification, as shown in a number of reports based on elegant studies in genetically engineered animals, which have been reviewed elsewhere [19,20].

**Role of uraemic toxicity**

CKD is characterized by the retention of uraemic solutes which are normally excreted by healthy kidneys. The uraemic syndrome is attributed to the progressive retention of a large number of compounds, called uraemic toxins, which are thought to interact negatively with numerous biological functions. The uraemic state represents a unique clinical condition in which direct tissue toxicity goes along with indirect toxic effects of retention solutes such as mineral and endocrine disturbances, inflammation and oxidative stress. In patients with advanced stages of CKD, both classical and non-classical cardiovascular risk factors have been considered to be of prime importance [21,22]. To evaluate the effects of the different classes of uraemic toxins in an optimal manner, they have been recently classified, providing a systematic analytical approach and mapping the relative importance of the enlisted families of toxins [23]. Many protein-bound molecules are small, water-soluble compounds of low-molecular weight (LMW), characterized by reduced removal during standard dialysis using small-pore membranes. Peptidic and non-peptidic substances of middle- and high-molecular weight also accumulate in uraemia, including inflammation and oxidative stress products, and are also considered as uraemic toxins [23]. Uraemic retention solutes may exert toxicity, especially if they are protein-bound [24]. The biological action of such compounds is nevertheless exerted only by their free fraction. Hence, the use of total blood or plasma concentrations for activity assessment in vitro is only adequate if the experimental medium contains appropriate amounts of plasma proteins.

In order to examine the effect of the uraemic state on vascular disease progression, we and others assessed the effects of chronic renal failure on vascular calcification and atherosclerosis in vivo, using the apolipoprotein-E (apoE−/−) knockout mouse model [25–27]. We found enhanced progression of both intimal and medial vessel wall calcification in uraemic mice, as compared with non-uraemic mice. In addition, we also observed accelerated atheromatous plaque formation [27], in agreement with two other groups [25,26], along with an increase in plaque collagen content. This uraemic mouse model was useful for subsequent experiments aimed at exploring in more detail the contribution of the uraemic state to the progression of atherosclerosis and calcification. We administered sevelamer for this purpose to apoE−/− mice with normal and reduced renal function, respectively [28]. As expected, we found that sevelamer treatment of uraemic apoE−/− mice reduced the progression of arterial calcification, in association with a significant decrease in serum phosphate and calcium phosphate product. Unexpectedly, sevelamer also delayed the progression of atherosclerosis, to become similar to the level of apoE−/− mice with normal renal function. This effect was observed in the absence of a change in serum total cholesterol levels. We could not, however, exclude possible changes of low-density (LDL) and/or high-density lipoproteins (HDL) in this study. We then examined the possible role of uraemic toxins. We failed to observe changes in the serum concentration of five compounds tested, although their serum levels were higher in uraemic than non-uraemic mice. On the other hand, the delayed progression of both vascular calcification and atherosclerosis in response to sevelamer treatment was associated with a significant decrease in the aortic expression of nitrotyrosine, a marker of local oxidative stress. In this study, we did not measure advanced oxidation protein products (AOPP) as markers of systemic oxidative stress. In any case, we believe that assessment of oxidative stress in target vascular tissue, wherever possible, is more relevant than circulating markers. As noted above, CKD is a state of chronic oxidative stress, with excessive production of reactive oxygen species and impaired anti-oxidant defence [29]. This is reflected by increased circulating levels of oxidative stress markers such as AOPP, which can be considered as high-molecular weight uraemic toxins and which are also active players by themselves [30]. Oxidative stress has also been shown to favour vascular calcification in vitro, by stimulating the transformation of VSMC to osteoblast phenotype. Thus, oxidized LDL have been shown to induce the calcification process in vitro [31–35]. This effect can be blocked by HDL [31]. In CKD patients, the reduction by sevelamer of serum total cholesterol, LDL cholesterol and C-reactive protein levels along with the reduced progression of arterial calcification are in favour of a beneficial effect of the induced changes in lipid metabolism and inflammation [36]. In dialysis patients, sevelamer has been shown to reduce serum levels not only of phosphate, but also of other LMW uraemic toxins such as uric acid in patients with CKD [37]. Moreover, in experiments in vitro it was able to absorb uraemic compounds such as indoxyl sulfate, indole and p-cresol [38]. Based on our results with sevelamer in this experimental model and on in vitro and in vivo findings by others, we propose the hypothesis that the effects of this phosphate binder on oxidative stress, and more generally speaking on uraemic toxicity, represents a therapeutic modality for cardiovascular disease, including arterial calcification, beyond the mere control of hyperphosphataemia (Figure 1).
Conclusion

Numerous in vitro and in vivo experiments have provided valuable novel information on the process of vascular calcification and related cardiovascular disease. They indicate potential targets and point to potential tools for future studies, aimed at halting the ectopic deposition of calcium and phosphate and possibly reversing this process. Among these targets, the uraemic syndrome with its direct and indirect toxicity for practically all tissues and biological systems deserves particular attention. A reduction of the retention of uraemic toxins of any type and molecular class, and of their widespread noxious effects, including substances which enhance oxidative stress and/or impair anti-oxidant defence, may help to decrease the incidence and progression of vascular calcification and related cardiovascular morbidity and mortality. Therefore, we believe that it is time to develop and to explore new pharmacological approaches to clear not only one uraemic toxin but several of them at the same time (Figure 1). This hypothesis, if confirmed by additional solid clinical and experimental evidence, might complement renal replacement therapy procedures in those CKD patients who are already on dialysis treatment, and represent a valuable treatment approach in those who have not yet reached end-stage kidney disease.

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References


IgA nephritis: ACE inhibitors, steroids, both or neither?

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Introduction

Immunoglobulin A nephropathy (IgAN), a disease characterized by the predominant deposition of IgA in the glomerular mesangium, is the most common glomerulonephritis in the world. No more than 10% of all patients experience complete remission of the urinary abnormalities, and IgAN frequently progresses towards chronic renal impairment, leading to end-stage renal disease in about 25% of the patients within 20 years [1]. Should this nephropathy be treated? If so, what is the best therapy? And should all patients be treated with the same drugs? The real problem is that, although some progress has been made in our pathogenetic understanding, the disease is still largely unknown and no specific treatment is available.

Given the variability of the clinical and histological manifestations of IgAN, it is perhaps not surprising