Vascular Calcification in Animal Models of CKD: A Review

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**Abstract**

Vascular calcification is a significant contributor to the cardiovascular mortality observed in chronic kidney disease (CKD). This review discusses the animal models (5/6 nephrectomy, mouse electrocautery model and dietary adenine) that have been employed in the study of vascular calcification outcomes in CKD. Rodent models of CKD generate a range of severity in the vascular calcification phenotype. Major limitations of the 5/6th nephrectomy model include the requirement for surgery and the need to use either excessive dietary phosphorus or vitamin D. Major limitations of the mouse electrocautery model include the requirement for surgery, the mortality rate when very advanced CKD develops, and resistance to vascular calcification without the use of transgenic animals. This is balanced against the major advantage of the ability to study transgenic animals to further understand the mechanisms associated with either the acceleration or inhibition of calcification. Dietary adenine generates severe CKD and does not require surgery. The major disadvantage is the weight loss that ensues when rats receive a diet containing 0.75% adenine. In summary, animal models are useful to study CKD-associated vascular calcification and the results obtained in these pre-clinical animal studies appear to translate to the evidence, however limited, which exists in humans with CKD.

**Key Words**

Chronic kidney disease · Cardiovascular mortality · Vascular calcification

**Rodent Models of Chronic Kidney Disease and Vascular Calcification**

Chronic kidney disease (CKD) is a worldwide health problem with a rising incidence and poor outcomes. Cardiovascular disease (CVD) is recognized as an important cause of morbidity and mortality in patients with CKD. In end-stage kidney disease (ESKD) patients, the presence and extent of arterial calcification is independently predictive of subsequent CVD and mortality beyond established conventional risk factors [1, 2]. New advancements in technology, despite their limitations, have increased our recognition of the extent of vascular calcification in patients with CKD; noninvasive techniques such as computed tomography have enabled us to quantify the degree of calcification. Studies consistently demonstrate that the majority of ESKD patients have significant calcification in the coronary arteries and aorta, and nearly 50% have valvular calcification [3].
Current techniques used clinically to quantify calcification lack the ability to distinguish between medial or intimal calcification. While calcification of the media and intima may occur simultaneously in the same patient, medial wall calcification is a consistent and early feature of CKD-associated vascular calcification. The pathophysiology of medial wall vascular calcification in CKD is complex and CKD-associated abnormalities in mineral metabolism (hyperphosphatemia, hyperparathyroidism, hypocalcaemia and vitamin D deficiency) contribute. Although observational studies have contributed to our understanding of the various risk factors associated with calcification in humans with CKD, there is a lack of randomized controlled trial evidence to indicate which risk factors predominate and which therapies might benefit patients most. Animal models, therefore, allow us to evaluate clinical observations made in humans within a controlled environment and, in doing so, will allow us to better understand the contributors to and the biological significance of vascular calcification in the setting of CKD. The objective of this review therefore is to discuss the animal models (5/6 nephrectomy, mouse electrocautery and adenine models) that have been employed to study vascular calcification outcomes in the setting of CKD.

Animal Models Have Contributed to Our Understanding that Vascular Calcification in the Setting of CKD Is an Active Process

Calcification occurs at two sites in the arterial wall: at the intima and the media. Intimal calcification is patchy, associated with vascular smooth muscle cells (VSMCs) and macrophages in lipid-rich areas of arteries and takes the form of atherosclerotic vascular disease. Intimal calcification has been associated with classic Framingham risk factors such as advancing age, diabetes, dyslipidemia, hypertension and smoking. In contrast, medial calcification occurs within the elastic regions of the arteries and almost exclusively associated with VSMCs [4]. Intimal and medial calcification may occur independently of each other and therefore are believed to represent different pathological processes. In young adult CKD patients, medial wall calcification predominates almost exclusively [5] and its presence has been linked to abnormalities in mineral metabolism that occur by stage 3 CKD (hyperphosphatemia) and its treatment (calcium-based phosphate binders and vitamin D).

There is growing in vitro and in vivo evidence that hyperphosphatemia and extracellular calcium stimulate phenotypic transformation of VSMCs [4, 6]. VSMCs have been found to exhibit distinctive phenotypes including a contractile phenotype characterized by markers of smooth muscle lineage (SMAD6, matrix Gla protein (MGP), α-smooth muscle actin) as well as phenotypes characterized by an osteochondrogenic-like differentiation and bone formation (sox-9, core-binding factor-α1, osteocalcin) [7, 8]. In the setting of elevated phosphorus levels, as occurs in CKD, the latter phenotype becomes more prevalent. A number of gene knockout experiments using animal models have confirmed that there are VSMC- and bone-derived proteins that either inhibit or promote the calcification process. Selective gene knockout models for bone-associated proteins such as osteoprotegerin [9] or selective gene knockout models of VSMC proteins such as smad6 [10] and MGP [11] all develop varying degrees of arterial calcification. Whether a final common pathway exists in the development of vascular calcification will require more extensive experimentation in animal models of CKD.

Animal CKD Models Develop Vascular Calcification

Research regarding the progression of changes in CKD has identified a remarkable consistency of phenotypes between animals and humans. Common to all models is an increased plasma creatinine, increased blood urea nitrogen, hyperparathyroidism and hyperphosphatemia. However, these rodent models of CKD generate a range of severity in the vascular calcification phenotype possibly due to a lack of consistency in genetic background, degree of kidney damage, time course of study, and dietary regimen.

Since the first publication in 1889, numerous investigators studied kidney disease by surgically reducing the kidney mass by 2/3 or 3/4 in various animal species. In 1932 Chauntin and Ferris [12] developed the 5/6 nephrectomy (5/6Nx) rat model which has been used ever since. The hallmark of all these studies is the development of uremia and CKD-related complications similar to the human condition. To the best of our knowledge, the first report of vascular calcification, using the 5/6Nx model, was published in 1979 [13]. Subsequently, the first mouse model of CKD reported by Gagnon and Duguid [14] in 1983 used a slightly different approach that continues to be employed by several groups to study vascular calcification outcomes. In this model, similar to 5/6Nx, most of
the mass of one kidney is reduced using electrocautery on the kidney surface followed by contralateral nephrectomy. A third model, first reported in 1982 [15] but which has received significant attention recently, is the induction of CKD using dietary administration of the renal toxin, adenine, in rats. While studying the metabolic fate of dietary purines, Yokozawa et al. [15] discovered that, in the setting of high dietary adenine, 2,8-hydroxyadename is formed and due to its low solubility in water it forms and precipitates along the tubules and urinary tract causing nephrotoxicity and the development of symptoms that are similar to clinical CKD.

Although there are inherent differences between these three models, each challenge generates a certain level of CKD after an initial acute insult to the kidneys, and the development of vascular calcification may or may not result in the following 4–36 weeks (tables 1–4). The degree of vascular calcification that develops within these models has typically been detected via tissue chemical analysis or chemical quantification of tissue calcium eluted in acid as follows by a qualitative tissue-staining procedure for calcium localization. In most cases tissue calcium is eluted in acid and the supernatant is measured using the cresolphthalein o-complexone method or atomic absorption. However, the digestion of tissue with acid results in the destruction of tissue, and whether increased calcium content is representative of actual calcification or just calcium excess requires confirmation via a von Kossa stain of previously fixed aortic sections. Although various visualization techniques have been used including Alizarin Red staining of whole tissue mounts, chemical quantification alone is insufficient to evaluate calcification of aortic tissue.

**The 5/6 Nephrectomy Model of CKD**

All variations of this model reduce the total mass of the kidney by 5/6th. The most common technique is a two-step procedure which requires removal of the two poles of one kidney (2/3rd of the first kidney) followed by full nephrectomy of the opposite intact kidney 1 week later. Table 1 refers to vascular calcification outcome studies that have compared the 5/6Nx animal to sham controls. The primary differences between studies have been the duration of CKD used in the study, and the dietary concentration of calcium and phosphate.

The 5/6Nx model has been found to produce serum creatinine levels which are on average 2.2-fold higher than control animals (table 1). Without the concurrent use of vitamin D, the 5/6Nx rats develop phosphorus levels 8 weeks after surgery that range up to 2.6-fold higher than control animals (with the exception of one study which reports a 3.8-fold increase), under dietary regimens containing between 0.9 and 1.2% phosphorus by weight. Based on the American Institute of Nutrition, the normal dietary phosphorus for rats is about 0.3% (depending on the formula used) [16]. Hyperparathyroidism is a feature of this model with studies reporting increases

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals</th>
<th>Duration after Nx, weeks</th>
<th>Diet</th>
<th>Groups</th>
<th>Serum Cr</th>
<th>Serum PTH</th>
<th>Serum P</th>
<th>Serum Ca</th>
<th>Serum Ca content</th>
<th>Von Kossa analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejerblad et al. [13], 1979</td>
<td>SD 200–334 g (n = 366)</td>
<td>12/36</td>
<td>2% Ca 1% P</td>
<td>Nx –12 weeks</td>
<td>1.8</td>
<td>1.7</td>
<td>1.0</td>
<td>0.92</td>
<td>NA</td>
<td>–/+</td>
</tr>
<tr>
<td>Haut et al. [47], 1980</td>
<td>SD rats (n = 54) Partial Nx or uni-Nx</td>
<td>18</td>
<td>0.5, 1, 2% P</td>
<td>Uni-Nx+ 2% P (n = 6)</td>
<td>2.3</td>
<td>NA</td>
<td>2.6</td>
<td>NA</td>
<td>1.65</td>
<td>NA</td>
</tr>
<tr>
<td>Cozzolino et al. [17], 2003</td>
<td>6-week-old SD rats (n = 97+)</td>
<td>24</td>
<td>0.6% Ca 0.9% P</td>
<td>Nxa</td>
<td>2.6</td>
<td>1.77</td>
<td>2.4</td>
<td>1.0</td>
<td>1.55</td>
<td>NA</td>
</tr>
<tr>
<td>Mizobuchi et al. [48], 2005</td>
<td>7-week-old SD rats (n = 52)</td>
<td>10</td>
<td>0.4% Ca 1.2% P</td>
<td>300 IU/kg Vit D</td>
<td>2.1</td>
<td>2.4</td>
<td>3.8</td>
<td>0.99</td>
<td>NA</td>
<td>+++</td>
</tr>
<tr>
<td>Gracioli et al. [49], 2009</td>
<td>300–350 g male Wistar (n = 47)</td>
<td>8</td>
<td>Regular chow with LP (0.2%) or HP (1.2%)</td>
<td>Nxa-LP</td>
<td>1.8</td>
<td>10.9</td>
<td>1.3</td>
<td>1.0</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nxa-Hp</td>
<td>2.4</td>
<td>0.63</td>
<td>3.2</td>
<td>1.50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nxa-hPTH-HP</td>
<td>1.23</td>
<td>1.28</td>
<td>4.72</td>
<td>1.53</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nxa-hPTH-LP</td>
<td>3.2</td>
<td>23.8</td>
<td>1.1</td>
<td>1.2</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

SD = Sprague-Dawley; Ca = calcium; P = phosphorous; LP = low phosphorous diet; HP = high phosphorous diet; Nx = nephrectomy; Cr = creatinine; PTH = parathyroid hormone; hPTH = supraphysiological level of PTH infusion; † = fold increase over control; ‰ = percent reduction over Nx.
between 2.4- and 77-fold over control animals, whereas serum calcium is minimally altered.

Ejerblad et al. [13], using 5/6Nx in Sprague-Dawley (SD) rats, were the first to report aortic calcification after 12–36 weeks using a 1% by weight phosphorus-containing diet (table 1). Interestingly, the data suggest that without a high phosphorus diet the commonly used SD rat strain was resistant to vascular calcification. For example, of the 8 studies that quantified aortic calcium content, in only 1 study [17] was there even a minor increase in aortic calcium content using a diet that contained less than 1% phosphorus (tables 1, 2). Therefore, either high dietary phosphorus (i.e. 1.2% phosphorus in the diet for periods of >12 weeks) or the use of an agent that promotes arterial calcification, such as 1,25(OH)2D3 (calcitriol), has become a common constituent of this model.

Table 2 has data summarizing the various studies that enable comparison of various treatment regimens (calcitriol, vitamin D analogs, calcimimetics and phosphate binders) in animals with CKD. In the 5/6Nx model, concurrent administration of vitamin D appears to result in circulating phosphorus concentrations that are about 1.8-fold increased over sham-treated animals and 1.3-fold increased over CKD animals not receiving vitamin D. Only mild elevations in calcium (<10%) are observed and yet there is a substantial reduction in parathyroid hormone (PTH) when compared to CKD animals not receiving concurrent 1,25(OH)2D3 (from 37 to 83% reduction).
With the administration of vitamin D (either calcitriol or an analog), vascular calcification appears to be accelerated in this animal model with at least a doubling of aortic calcium content and in 1 case a 28-fold increase over CKD animals not receiving vitamin D [18]. In general, if one also considers those studies which only evaluated calcification with von Kossa staining (i.e. the investigators did not determine vessel calcium content), it is evident that, in the 5/6Nx CKD animal model, the vitamin D-treated animals consistently demonstrate greater severity of calcification without great perturbations in phosphate concentrations.

The major limitations of using the 5/6Nx model for the study of vascular calcification have been the requirement of a two-step surgical procedure and the need to use either excessive dietary phosphorus or an accelerating agent such as vitamin D. Further, even with a reduction of 5/6th of the renal tissue, SD rats are not prone to develop severe kidney damage, and as a result these animals do not routinely calcify in studies lasting less than 12 weeks after surgery (table 1), even with dietary phosphorus less than 1%. That is, without high dietary phosphorus (≥1.2%) or supplementary calcitriol treatment young SD rats do not appear to develop calcification unless CKD is substantially prolonged.

**Vitamin D Analogs, Phosphate Binders, and Calcimimetics Reduce Vascular Calcification in the 5/6Nx Model to CKD**

For decades, calcitriol has been used to treat hyperparathyroidism; however, in the 5/6Nx animal model, the administration of calcitriol promotes hyperphosphatemia, mild hypercalcemia, and increases the prevalence of vascular and tissue calcification (table 2). New vitamin D analogs or calcimimetics (22-oxacalcitriol, paricalcitol, cinacalcet, R-568 and AMG 641) have been developed to minimize the development of hypercalcemia while treating hyperparathyroidism as effectively as calcitriol. However, no clinical trial in humans has compared calcification outcomes between these newer vitamin D analogs and calcitriol; the only comparative studies have been performed in 5/6Nx animal models and consistently demonstrate the adverse effect of calcitriol on calcification in this model (table 2).

In 2003, Hirata et al. [19] compared the effect of calcitriol (0.125 µg/kg/day) and 22-oxacalcitriol (vitamin D analog, 6.25 µg/kg/day) on serum PTH and aortic calcification in 5/6Nx rats. Although both agents lowered PTH similarly, calcitriol produced a 7-fold increase in aortic calcium content whereas there was no effect in the 22-oxacalcitriol treatment group. Similar results were reported by Wu-Wong et al. [20], Cardús et al. [18], and Lopez et al. [21], all of whom compared the vitamin D analog paricalcitol with calcitriol. There was either no increase in aortic calcium content with paricalcitol over CKD controls [20] or a minimal increase in calcification [18, 21] compared to the substantive impact seen in the calcitriol-treated groups. Henly et al. [22] compared calcitriol (0.25–0.28 µg/kg/day) to the calcimimetic, cinacalcet, and reported significantly increased calcification in all animals that received any calcitriol treatment. Interestingly, concomitant treatment with a calcimimetic (cinacalcet) reduced vascular calcification.

Other studies have employed the 5/6Nx model plus concurrent calcitriol treatment to study the effect of bisphosphonates and ammonium chloride on the progression of vascular calcification. In one study calcitriol (1 µg/kg/day) was given to 5/6Nx animals; however, the primary purpose was to investigate the dose-response effect of the bisphosphonate, etidronate, on vascular calcification [23]. In another study the effects of metabolic acidosis (ammonium chloride) on vascular calcification was studied using 5/6Nx animals on calcitriol (1 µg/kg/day) [24]. Both groups were able to produce extensive accelerated calcification within 2–5 weeks, and consequently demonstrated a reduction in calcification with etidronate or ammonium chloride treatment respectively.

**Mouse Electrocautery Model of CKD**

The CKD model in mice using surface electrocautery of the kidney was developed by Gagnon and Duguid [14] in 1983. More recently this approach has been combined with transgenic mouse models to promote vascular calcification in the setting of either the metabolic syndrome using the low density lipoprotein receptor knockout mouse (LDLR−/−), or accelerated atherosclerosis (apolipoprotein E knockout (apoE−/−)); table 3). In the mouse electrocautery model, CKD is induced by surgical ablation of the kidneys. This is a two-step procedure; initially the cortex of one kidney is electrocauterized paying careful attention not to destroy the adrenals and the hilum of the kidney. One week later, once the animals have recovered, the second kidney is nephrectomized. This procedure appears to produce variable severity of CKD with blood
LDLR−/− mice without CKD have a predisposition to atherosclerosis, insulin resistance and early type 2 diabetes. The abolic syndrome as these mice have obesity, hypertension, insulin resistance and early type 2 diabetes. The LDLR−/− mice without CKD have a predisposition to accelerated vascular calcification and atherosclerosis on a high fat diet; however, the addition of CKD produces a 1.5- to 2.6-fold increase in aortic calcium content (table 3). Similarly, the apoE−/− mice fed a regular diet generate accelerated atherosclerosis in the form of increased intimal disease as well as medial calcification that becomes modestly elevated when CKD is induced in these animals.

Vascular calcification has not been consistently reported in wild-type mice with CKD. However, extensive calcification develops in LDLR−/− and apoE−/− transgenic mice using this electrocautery model. The LDLR−/− CKD model resembles clinical uremia with an associated metabolic syndrome as these mice have obesity, hypertension, insulin resistance and early type 2 diabetes. The LDLR−/− mice without CKD have a predisposition to accelerated vascular calcification and atherosclerosis on a high fat diet; however, the addition of CKD produces a 1.5- to 2.6-fold increase in aortic calcium content (table 3). Similarly, the apoE−/− mice fed a regular diet generate accelerated atherosclerosis in the form of increased intimal disease as well as medial calcification that becomes modestly elevated when CKD is induced in these animals.

A major limitation of the mouse electrocautery model, similar to the 5/6Nx model, is the requirement for surgery which can initially lead to complications. However, once a consistent surgical procedure has been developed, this is a reproducible model and relative homogeneity with respect to the degree of CKD can be achieved. Where reported, mortality ranges between 11 and 30% and is in part due to surgery and anesthesia as well as the complications when advanced CKD develops. The major advantage of using mice is the ability to study transgenic animals to further understand the mechanisms associated with either the acceleration or inhibition of calcification.

Table 3. Summary of studies using the mouse electrocautery model

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals</th>
<th>Duration after Sx weeks</th>
<th>Diet</th>
<th>Groups</th>
<th>BUN Serum</th>
<th>PTH Serum</th>
<th>P Serum</th>
<th>Ca Serum</th>
<th>Aortic Ca content</th>
<th>Von Kossa analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davies et al. [34], 2003</td>
<td>10 weeks LDLR−/− (n = 59)</td>
<td>18</td>
<td>High fat diet</td>
<td>CKD</td>
<td>↑ 3.5</td>
<td>NA</td>
<td>NA</td>
<td>↑ 1.3</td>
<td>↑ 2.6</td>
<td>++</td>
</tr>
<tr>
<td>Davies et al. [35], 2005</td>
<td>10 weeks male/female LDLR−/− (n = 64)</td>
<td>14</td>
<td>High fat diet</td>
<td>CKD</td>
<td>↑ 2.3</td>
<td>↑ 3.5</td>
<td>↑ 1.5</td>
<td>0.92</td>
<td>↑ 6.0</td>
<td>NA</td>
</tr>
<tr>
<td>Massy et al. [51], 2005</td>
<td>8 weeks Apo E−/− (n = 48)</td>
<td>6</td>
<td>Regular chow</td>
<td>CKD</td>
<td>↑ 3.6</td>
<td>↑ 3.6</td>
<td>↑ 1.1</td>
<td>↑ 1.1</td>
<td>NA</td>
<td>++</td>
</tr>
<tr>
<td>Mathew et al. [29], 2007</td>
<td>14 weeks LDLR−/− (n = 50)</td>
<td>14</td>
<td>High fat diet</td>
<td>CKD</td>
<td>↑ 3.3</td>
<td>↑ 2.6</td>
<td>↑ 1.1</td>
<td>↑ 1.1</td>
<td>NA</td>
<td>++</td>
</tr>
<tr>
<td>Mathew et al. [29], 2007</td>
<td>14 weeks C57 Black WT CKD</td>
<td>14</td>
<td>High fat diet</td>
<td>CKD</td>
<td>↑ 3.6</td>
<td>↑ 7.0</td>
<td>↑ 1.1</td>
<td>↑ 1.1</td>
<td>NA</td>
<td>++</td>
</tr>
<tr>
<td>Ivanovski et al. [35], 2005</td>
<td>8 weeks Apo E−/− (n = 31)</td>
<td>14</td>
<td>Regular chow</td>
<td>CKD</td>
<td>↑ 3.0</td>
<td>↑ 3.2</td>
<td>↑ 1.0</td>
<td>↑ 1.1</td>
<td>NA</td>
<td>++</td>
</tr>
<tr>
<td>Ivanovski et al. [53], 2009</td>
<td>8 weeks old female apoE−/−</td>
<td>10</td>
<td>NA</td>
<td>CKD</td>
<td>↑ 2.6</td>
<td>↑ 2.8</td>
<td>↑ 1.0</td>
<td>↑ 1.2</td>
<td>NA</td>
<td>++</td>
</tr>
<tr>
<td>Maizel et al. [54], 2009</td>
<td>8 weeks female C57B WT or ApoE−/−</td>
<td>6–10</td>
<td>0.99% Ca, 0.65% P</td>
<td>WT CKD – 6 weeks</td>
<td>↑ 3.4</td>
<td>↑ 1.0</td>
<td>↑ 1.1</td>
<td>↑ 1.1</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Alkawa et al. [26], 2009</td>
<td>20 weeks Apo E−/− and either catS−/− or catS+/+ (3/4Nx)</td>
<td>10</td>
<td>42% fat Atherogenic diet</td>
<td>CKD catS+/+</td>
<td>2.4</td>
<td>1.8</td>
<td>1.2</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>CKD catS−/−</td>
<td>1.7</td>
<td>1.4</td>
<td>0.9</td>
<td>+</td>
<td>+</td>
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</table>

LDLR = Low density lipoprotein receptor; ApoE−/− = apolipoprotein E knockout mouse; C57B WT = C57Black wild-type; CKD = chronic kidney disease; BMP-7 = bone morphogenic protein 7; CaCO3 = calcium carbonate; BUN = blood urea nitrogen; PTH = parathyroid hormone; P = phosphorous; Ca = calcium; WT = wild-type; catS−/− = cathepsin S knockout mouse; † = fold increase over control; ‡ = percent reduction over CKD.
Recently two new transgenic mouse CKD models have been developed to study the impact of either fetuin-A or cathepsin S gene deletion on soft tissue calcification. Fetuin-A is a major circulatory inhibitor of soft tissue calcification. Westenfeld et al. [25] developed the fetuin-A knockout CKD mouse model on the C56BL/6 genetic background. They did not detect vascular calcification in the CKD animals even when combined with the fetuin-A deletion. However, they did find calcification within the heart valves and myocardial tissue. On the other hand, Aikawa et al. [26] used the apoE−/−, cathepsin S deficient mice (catS−/−), and induced 3/4Nx surgically to develop CKD. They discovered that deletion of the elastase cathepsin S protected against vascular as well as valvular calcification in the apoE−/− CKD mice (table 3).

**Dietary Phosphate Binders and Bone Morphogenic Protein-7 Reduce Vascular Calcification in the Electrocautery Mouse Model**

In this model, phosphorus binders, whether calcium-based (i.e. calcium carbonate) or non-calcium-based (i.e. sevelamer), appear to lower phosphorus levels (range from 28 to 42% reduction) and demonstrate a consistent, decrease in aortic calcium content when compared to untreated CKD animals in both LDLR−/− and apoE−/− knockouts [27–29]. Interestingly, the phosphate binder sevelamer is capable of reducing intimal calcification in apoE−/− CKD mice and therefore may also prevent atherosclerosis progression in this model [27]. It is well known that sevelamer reduces cholesterol levels in humans suggesting that a non-phosphorus lowering benefit of this drug may exist [30–32]. The findings in the electrocautery mouse model are consistent with the 5/6Nx and adenine models of CKD [17, 33] (tables 2, 3).

Davies et al. [34, 35] have investigated the link between low turnover osteodystrophy and vascular calcification in LDLR−/− mice with CKD receiving a high fat diet. bone morphogenic protein-7 (BMP-7) is an important regulator of bone remodeling and development. It restores bone anabolic balance by stimulating bone formation and reduces serum phosphorus via increased uptake into bone. Treatment with BMP-7 lowered serum phosphorus and prevented CKD-induced vascular calcification. Whether reduced serum phosphorus is achieved by a phosphate binder (calcium-based or sevelamer), or agents that stimulate bone formation (BMP-7), the result is reduced vascular calcification in this mouse model of CKD.

**Adenine Rat Model**

The adenine model has recently gained attention due to its relative ease of design and encouraging outcomes. Within 4 weeks on a 0.75% adenine diet, calcification of the tunica media of the aorta has been reported, and combined with a low protein diet, the calcification outcome is more consistent [36]. This model, unlike the other models, does not require surgery, and has a high survival rate. As demonstrated in table 4, following 4 weeks of an adenine diet, the creatinine levels are 3.0- to 10.5-fold higher than control animals. Serum phosphorus levels range from 1.2- to 2.6-fold higher than normal with severe secondary hyperparathyroidism despite minimal fluctuations in the serum calcium (table 4).

With dietary phosphorus concentrations ranging from 0.4 to 1.2%, the adenine model produces the highest serum phosphorus levels, likely due, in part, to the severity of kidney damage. With the severity of CKD produced in this model, vascular calcification is consistently generated without additional calcitriol (as in the 5/6Nx model) or a genetic predisposition to calcification (as in the mouse electrocautery model). However, in those studies which report weight loss, rats fed 0.75% adenine lost up to 50% of their initial weight within 5 weeks on the diet [36–38]. Whether the extensive medial calcification observed is due, in part, to weight loss and volume contraction or the adenine itself remains to be determined.

The adenine model has been used to study the role of the phosphate binder sevelamer and bisphosphonates (ibandronate, etidronate, pamidronate and alendronate) in modifying the vascular calcification phenotype (table 4). Compared to animals with CKD receiving adenine alone, these treatments resulted in significant reductions in aortic calcium content (99% reduction with ibandronate and 84% reduction with sevelamer).

The major advantage of the adenine model is that it does not require surgery to generate severe CKD. The major disadvantage is the weight loss that ensues when rats receive a diet containing 0.75% adenine. However, in a recent report of a modified version of the adenine model, weight loss was not an issue. Instead of using a chow-based adenine diet, Terai et al. [39] orally dosed adenine at 600 mg/kg/day for 10 days as the initial acute insult to induce CKD in Wistar rats. On a normal diet with or without high phosphate (i.e. no adenine in the diet) these rats grew steadily over the 15-week study period and developed progressive renal failure. After 8 weeks of CKD only 12.5% had vascular calcification, but after 15 weeks, 37.5% of the animals developed vascular calcification.
Thus far we have discussed three mild to moderate CKD models that have addressed the abnormal serum biochemistries (Ca, P, PTH and vitamin D) and bone abnormalities (i.e. the LDLR<sup>–/–</sup> CKD model) that accelerate vascular calcification. However, the CKD models so far discussed occur following an acute injury to the kidney which then develops persistent CKD. A recent report by Moe et al. [40] describes spontaneous development of CKD over time in rats with autosomal dominant polycystic kidney disease. Heterozygous Han:SPRD rats (Cy/+ ) develop azotemia at about 10 weeks of age and progress to uremia by about 40 weeks. These animals develop persistent elevated BUN at 20 weeks as well as increased creatinine, reduced body weight and anemia by week 38. On a normal phosphorus diet, these animals develop progressive hyperphosphatemia, hyperparathyroidism and bone abnormalities; however, medial calcification is not progressive and is only evident in 60% of the older animals by week 38. This model provides the advantage of spontaneous CKD but at the expense of a long duration of study. This model may, however, prove most useful for the study of preventive strategies. Also of interest is the report of Persy et al. [41] who successfully demonstrated in vivo calcification of living rats using high-resolution X-ray microtomography. Their method is reproducible and allows quantification of calcification without the need of ex vivo analysis, which could provide future studies a new perspective in designing interventions (tables 1–3).
Comparing Studies in Animal Models to Clinical Trials in Humans with CKD

There are few randomized controlled trials studying vascular calcification outcomes in humans with CKD despite the documentation of the extent of the clinical problem. The Treat-to-Goal study [42] compared two dietary phosphate binders (sevelamer versus calcium-based phosphate binders) in patients with ESKD. Consistent with the findings in the mouse electrocautery model [27, 29] and the adenine model [33], humans with ESKD randomized to sevelamer had a slower rate of accumulation of calcium in the coronary arteries and the aorta after 1 year of treatment. These results have been further corroborated in a study examining incident dialysis patients [43]. In both of these trials, treatment with calcium-based phosphate binders appears to enhance calcification.

The role of vitamin D in either the promotion or prevention of calcification in CKD patients remains controversial. In the 5/6Nx model, the administration of calcitriol clearly accelerates tissue calcium deposition, a finding which is somewhat ameliorated with vitamin D analogs. We found that the use of vitamin D was associated with either the absence of or only mild increases in calcium levels, the purported mechanism via which vitamin D may accelerate vascular calcification. Therefore, the results of these animal studies suggest that vitamin D may have a direct effect on the vascular smooth muscle cell itself. Exploring this concept further in animal models is critical to the care of kidney patients given the frequency with which patients with ESKD are prescribed vitamin D preparations.

The role of calcimimetics in modifying coronary artery calcification in humans is currently under randomized controlled study [44]. Preclinical data demonstrating benefit is only just emerging, but a published human study evaluating its role in calcification is lacking. As demonstrated in the 5/6Nx model [21, 22, 45] there may be a role for this calcium-sensing receptor antagonist in ameliorating vascular calcification in humans possibly through its well-described ability to lower serum calcium and phosphate or perhaps through its potential to upregulate MGP, the VSMC protein considered the most important local inhibitor of vascular calcification.

Two animal studies (5/6Nx and adenine) have demonstrated that bisphosphonates ameliorate calcification [23, 36]. Preliminary evidence in humans with CKD confirms these findings and randomized controlled trials evaluating this outcome are in progress [46].

Conclusion

In conclusion, animal models are useful to study CKD-associated vascular calcification and the results obtained in these preclinical animal studies appear to translate to the evidence, however limited, that exists in humans with CKD. Transgenic mouse models have proved their usefulness in studying potential mechanisms underlying the biologic process while studies in rats are useful to study the natural history of the process and the treatments that may modify it. By taking observations made in humans in CKD and subjecting those observations to controlled experimentation in animals, we may be able to better understand and control the epidemic of CVD in patients with kidney disease.

References


CKD Animal Models of Vascular Calcification

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