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Vascular calcification: Contribution of parathyroid hormone in renal failure

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Hyperphosphatemia is a driving force in the pathogenesis of vascular calcification (VC) and secondary hyperparathyroidism associated with renal failure. To test for the possible contribution of parathyroid hormone (PTH) to cardiovascular calcification, we removed the parathyroid glands from rats but infused synthetic hormone at a supraphysiologic rate. All rats were pair-fed low, normal, or high phosphorus diets and subjected to a sham or 5/6 nephrectomy (remnant kidney). Control rats were given a normal diet and underwent both sham parathyroidectomy and 5/6 nephrectomy. Heart weight/body weight ratios and serum creatinine levels were higher in remnant kidney rats than in the sham-operated rats. Remnant kidney rats on the high phosphorus diet and PTH replacement developed hyperphosphatemia and hypocalcemia along with low bone trabecular volume. Remnant kidney rats on the low phosphorus diet or intact kidney rats on a normal phosphorus diet, each with hormone replacement, developed hypercalcemia. All rats on PTH replacement developed intense aortic medial calcification, and some animals presented coronary calcification. We suggest that high PTH levels induce high bone turnover and medial calcification resembling Mömckeberg's sclerosis independent of uremia. This model may be useful in defining mechanisms underlying VC.

Kidney International (2007) **71,** 1262–1270; doi:10.1038/sj.ki.5002241; published online 4 April 2007

KEYWORDS: phosphorus; renal osteodystrophy; vascular calcification; kidney failure; chronic; parathyroid hormone

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Received 24 March 2006; revised 12 January 2007; accepted 14 February 2007; published online 4 April 2007

Cardiovascular diseases are the leading causes of death in patients with chronic kidney disease (CKD) and account for at least 50% of deaths in this population,[1] especially among those undergoing dialysis.[2,3] Although there is undoubtedly an excess of cardiovascular mortality, the factors involved have not been conclusively identified. One likely factor is vascular calcification (VC). In CKD patients, histologic and radiographic evidences of VC are markedly higher than in the general population[4] and has been shown to have a significant adverse influence on a number of surrogate markers for cardiovascular disease mortality. Several explanations for this striking degree of VC observed in CKD patients have been proposed, including deregulation of the calcium/phosphate metabolism implicit in renal osteodystrophy and the influence of CKD treatment with calcium-containing phosphate binders or vitamin D analogs.[4]

The importance of managing hyperphosphatemia has classically been emphasized because of its role in the pathogenes of secondary hyperparathyroidism.[5] However, disturbances in phosphorus metabolism and parathyroid hormone (PTH) have other significant adverse consequences.[6] In recent studies, hyperphosphatemia, alone or in combination with the measures currently used to control it, has been implicated in the substantially higher incidence of cardiovascular mortality, as well as in the higher incidence of visceral and peripheral VC, seen in patients with advanced CKD.[7] Although the exact mechanism is unknown, VC may be involved. Jono *et al.*[8] showed, *in vitro*, that inorganic phosphate transformed vascular smooth muscle cells into calcifying cells by a direct mechanism involving the phosphate cotransporter Pit-1. Hyperphosphatemia-related hypersecretion of PTH may also be indirectly involved.[9] As for the exact effects that PTH has on VC, this is still a matter of debate. Recently, high PTH serum levels were associated with higher mortality rates in dialysis patients.[6,10] However, there have been some *in vitro* studies in which a direct effect of PTH on VC could not be confirmed[11] and others in which it was argued that PTH treatment of osteoporosis can also decrease VC progression.[12] Finally, an isolated PTH or phosphorus effect cannot be evaluated in CKD patients because hyperphosphatemia is typically accompanied by elevated PTH and other factors that could interfere with their effects. In addition, many patients receive treatment for

hyperphosphatemia or hyperparathyroidism that could itself facilitate VC, worsen renal function, and adversely affect bone remodeling. Using an experimental model of chronic renal failure with continuous infusion of a physiological concentration of PTH, we recently demonstrated that isolated hyperphosphatemia was accompanied by myocardial hypertrophy, worsening of renal function, and negative effects on bone remodeling.[13] However, in that study, we observed no VC, even in the presence of severe hyperphosphatemia.

In this study, we designed an experimental model involving diets with high phosphorus content that would induce hyperphosphatemia or low phosphorus content to control phosphorus levels, as well as measures to ensure constant, high levels of PTH.[14] We did so to investigate the isolated effect of PTH on cardiovascular calcification, cardiac fibrosis, other cardiovascular changes, renal function, and bone, in animals with chronic experimental uremia as well as in those with normal renal function.

RESULTS

Body weight, food intake, tail cuff plethysmography, and heart weight/body weight ratio

Table 1 and Figure 1 summarize the animal data. The duration of the uremia and diet-treatment experiments was 52 days. Initial body weight did not differ among the groups. Despite the pair-feeding protocol, LP + hi-PTH + Nx rats had the lowest final body weights. All Nx rats presented slightly lower food intake than did those with normal renal function. Initial tail cuff plethysmography readings were similar among all groups. After 8 weeks of uremia, all Nx rats developed hypertension, as did CP + hi-PTH rats. Regarding the heart weight/body weight ratio (HW/BW), Nx animals developed myocardial hypertrophy.

Biochemical findings

In Table 2, Figures 1 and 2, biochemical findings from all animals.

Hematocrit levels were higher in CP + SHAM rats. The Nx rats presented higher serum magnesium levels, although the difference was only statistically significant for LP + hi-PTH + Nx rats. The Nx rats developed moderate renal failure and proteinuria. The HP + hi-PTH rats presented lower creatinine clearance than their corresponding controls (CP + hi-PTH) and CP + SHAM rats. Reposition of PTH was similar among all hi-PTH groups. The HP + hi-PTH + Nx rats presented very low iCa levels and developed pronounced hyperphosphatemia, as evidenced by a marked elevation in calcium × phosphorus (Ca × P) product – even in the presence of hypocalcemia. The LP + hi-PTH + Nx and the CP + hi-PTH rats developed significant hypercalcemia. However, there were no differences in Ca × P product among the hi-PTH groups.

Morphological results

Tables 3 and 4 and Figure 1 show the results of the bone histomorphometric analysis. All hi-PTH rats developed features of hyperparathyroidism bone disease, presenting higher bone formation and resorption accompanied by medullar fibrosis. Trabecular volume and osteoid surface area were greater in LP + hi-PTH + Nx rats than in HP + hi-PTH + Nx rats. Trabecular separation was higher in the HP + hi-PTH + Nx animals than in their LP + hi-PTH + Nx counterparts, denoting an osteoporotic effect of the HP diet in uremic rats. Although the differences were not statistically significant, HP + hi-PTH and CP + hi-PTH rats presented higher bone formation rate/bone surface and lower mineralization lag time than did Nx rats.

Qualitative analysis of myocardial, renal, and arterial histology revealed that all hi-PTH rats developed intense aortic medial calcification. Macroscopically, the aortic artery had a 'tram-line' appearance resembling Mönckeberg's sclerosis. The diffuse mineral deposition occurred in the internal elastic lamina, particularly surrounding fractured disorganized elastin fibers. The medial vascular smooth muscle cells acquired cuboid aspects. The VC pattern was comparable among all hi-PTH groups. No intimal calcification was seen (Figure 3). Some animals also presented coronary medial calcification, and one rat in the HP + hi-PTH + Nx group also presented medial arterial calcification in the renal artery. Sparse calcium deposits in myocardial and kidney tissue were detected in some hi-PTH rats. Perivascular fibrosis was seen in the myocardial tissue of hi-PTH rats (Figure 4).

DISCUSSION

This study showed that rats receiving continuous infusion of high concentrations of PTH develop massive aortic medial calcification resembling Mönckeberg's sclerosis, as well as coronary medial calcification. These findings were apparently unrelated to the differences in dietary phosphorus or the

Table 1 | Animal data

	Initial BW (g)	Final BW (g)	Food intake (g/day)
HP+hi-PTH+Nx (*n*=7)	336.4 ± 8.5	340.1 ± 20.8	11.9 ± 0.9[b]
LP+hi-PTH+Nx (*n*=9)	316.0 ± 3.2	305.9 ± 7.3[a]	12.3 ± 0.29[b]
HP+hi-PTH (*n*=6)	342.7 ± 6.4	385.3 ± 15.5	16.0 ± 0.65
CP+hi-PTH (*n*=5)	350.5 ± 5.5	410.6 ± 8.9	16.3 ± 0.42
CP+SHAM (*n*=10)	328.7 ± 6.2	377.6 ± 13.2	15.6 ± 0.33

BW, body weight; CP, control phosphorus diet; hi-PTH, high PTH; HP, high phosphorus diet; LP, low phosphorus diet; Nx, nephrectomized rat; PTH, parathyroid hormone; SHAM, sham-operated.
[a]$P < 0.05$ vs all; [b]$P < 0.05$ vs Non-Nx rats.