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Mechanisms of Vascular Calcification in Uremia

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Vascular calcification and cardiovascular disease mortality are highly correlated with increased serum phosphate levels in end-stage renal disease patients. Mechanistic studies in cultured human smooth muscle cells (SMCs) indicate that increased phosphate levels induces both calcification and phenotypic transition through a pathway requiring a sodium-dependent phosphate cotransporter. Thus, in addition to contributing to increased calcium × phosphate product (Ca × P), hyperphosphatemia may have direct effects on SMCs that predispose these cells to calcium deposition in end-stage renal disease patients.
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The risk for cardiovascular disease mortality in end-stage renal disease patients is 20 to 30 times higher than in the general population.[1] Hyperphosphatemia has been linked to the increased cardiovascular mortality risk observed in dialysis patients in a growing number of studies.[2-4] Furthermore, both hyperphosphatemia and increased Ca × P promote vascular calcification.[2] Vascular calcification is correlated positively with atherosclerotic plaque burden,[5,6] increased risk for myocardial infarction,[7,8] increased ischemic episodes in peripheral vascular disease,[9] and is associated strongly with arterial stiffness, pulse pressure, and mortality in dialysis patients that likely further contributes to the high rates of cardiac and peripheral ischemic disease and left ventricular hypertrophy in this population.[10-12] Taken together, these findings suggest that hyperphosphatemia may increase the risk for cardiovascular death in dialysis patients by increasing vascular calcification. Understanding the mechanisms by which phosphate increases vascular calcification is thus of great importance.

Materials and Methods

Human smooth muscle cells (SMCs) were derived from autopsy specimens and used between passages 8 to 12. Ca deposition was determined by the o-cresolphthalein complexone method, and normalized to protein content as previously described.[13] Inorganic phosphate (Pi) uptake, polymerase chain reaction, RNA interference, and Northern blot analyses were performed using standard techniques.

Results

We hypothesized that vascular SMCs might respond to increased extracellular Pi levels by increasing promineralizing molecules, thereby leading to vascular calcification. To test this hypothesis, we treated SMC cultures with increasing concentrations of Pi. Under normophosphatemic conditions (1.4 mmol/L Pi), no culture mineralization was observed. In contrast, as Pi levels increased to hyperphosphatemic levels (2 mmol/L Pi) calcification was increased. By light and electron microscopy, mineralization was shown to be associated predominantly with extracellular matrix, particularly collagen, and consisted of bioapatite.[13]

Treatment of SMCs with 2 mmol/L Pi also induced a unique phenotypic transition characterized by increased expression of osteocalcin and Cbfa-1 messenger RNAs, both important in bone development.[14] As determined by Northern blot analysis, osteocalcin and Cbfa-1 messenger RNAs were increased as early as 24 hours after treatment with 2 mmol/L Pi.[13] A similar phenotypic transition was observed in medial cells in calcified arteries from matrix gla-protein null mice that expressed increased levels of osteopontin and Cbfa-1, and decreased α-smooth muscle actin levels, compared with SMCs in noncalcified wild-type vessels.[15]

To determine the mechanism by which SMCs sense increased Pi levels, we examined Pi uptake. Pi uptake in SMCs was sodium- and Pi-gradient dependent. Affinity constant (Km) of Pi transport was 1.53 ± 0.03 mmol/L and maximal

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velocity (Vmax) was 34.5 ± 0.8 nmole/mg protein. These properties are consistent with the presence of a sodium-dependent phosphate cotransporter (NPC) in SMCs. To determine whether NPC function was important in SMC culture calcification, we used the NPC inhibitors, phosphonoformic acid and arsenate. In the presence of phosphonoformic acid and arsenate, SMC Pi uptake as well as mineralization were blocked. In addition, phosphonoformic acid blocked up-regulation of osteocalcin and Cbfa-1 messenger RNA in increased phosphate-treated SMCs.[13] These studies point to a previously unappreciated role for a NPC in SMC mineralization as well as phenotype transition. In recent experiments, the RNA interference approach was used to definitively implicate Pit-1, a member of the type III NPC family, as the NPC important for driving human SMC mineralization in response to increased phosphate (Giachelli and Li, unpublished observations).

Conclusions

These data suggest that, in addition to increasing Ca × P, increased Pi may stimulate human SMCs directly to undergo phenotypic changes that predispose to calcification. The NPC, Pit-1, is required for these activities. These studies may help explain the phenomena of human metastatic calcification under hyperphosphatemic conditions as seen in the end-stage renal disease patient.

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