The Molecular Biology and Pathophysiology of Vascular Calcification

DOI: 10.3810/pgm.2014.03.2740

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Abstract: Vascular calcification (VC), commonly encountered in renal failure, diabetes, and aging, is associated with a large increase in the risk for cardiovascular events and mortality. Calcification of the arterial media and of heart valves clearly plays a mediating role in this regard, whereas it is less clear how calcification of plaque influences atherogenesis and risk for plaque rupture. Vascular calcification is an active process in which vascular smooth muscle cells (VSMCs) adopt an osteoblastic phenotype and deposit hydroxyapatite crystals; apoptosis of VSMCs also promotes this deposition. Drivers of this phenotypic transition, which include elevated serum phosphate, advanced glycation end-products, bone morphogenetic proteins, inflammatory cytokines, and leptin, invariably induce oxidative stress in VSMCs, which appears to be a necessary and sufficient condition for induction of the runt-related transcription factor 2 gene (*RUNX2*) and the shift to osteoblastic behavior. Magnesium antagonizes the impact of phosphate on VSMC osteoblastic transition, both by a direct effect within VSMCs and by suppressing absorption of dietary phosphate. Antioxidants that suppress reduced nicotinamide adenine dinucleotide phosphate oxidase activity may have the potential to block the osteoblastic transition of VSMCs. Minimizing the absorption of dietary phosphate may also be helpful in this regard, particularly in renal failure, and it can be achieved with plant-based dietary choices, avoidance of phosphate additives, and administration of pharmaceutical phosphate binders, supplemental magnesium, and niacin. Good vitamin K status opposes VC by optimizing the γ-carboxylation of matrix Gla protein, a physiological antagonist of VC. Adequate but not excessive vitamin D status also appears to discourage VC. Etidronate, a structural analogue of pyrophosphate, has shown potential for blocking VC.

Keywords: vascular calcification; oxidative stress; phosphate; vitamin K

An Osteoblastic Phenotypic Shift

The phenomenon of vascular calcification (VC), which includes calcification of intimal atheromatous plaque, medial calcification (arteriosclerosis), and calcification of the aortic valve or mitral annulus, is a frequent consequence of chronic renal failure, diabetes, and aging, and has been linked epidemiologically with an increased risk for cardiovascular events and mortality, independent of traditional risk factors.[1–3] A meta-analysis found that individuals with calcification in any vascular wall, compared with those who have no calcification, were at a 3- to 4-fold greater risk for cardiovascular events and cardiovascular and all-cause mortality.[3] Although VC can be serving as a marker for vascular inflammation and for the pathologies that provoke it, VC may also be pathogenic in its own right. The arterial stiffening associated with medial calcification promotes systolic hypertension, increased pulse pressure, and ventricular hypertrophy.[4] Aortic stenosis reflecting calcification of the aortic valve is one of the most common cardiovascular disorders, placing a major load on the heart and often requiring surgical correction.[5] Mitral annular calcification has been linked to

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an increased risk for atrial fibrillation and stroke, as well as increased cardiovascular mortality; the extent to which these associations are causal requires further clarification.[6–9] The calcification of arterial plaque is associated with an increased risk for plaque rupture and myocardial infarction; whether the calcification per se promotes this outcome, or is rather just a marker for the inflammatory status of plaque, remains unclear. Plaques with a spotty pattern of calcification appear to be less stable than those with extensive calcification.[10] It has been proposed that calcium phosphate crystals might destabilize plaque by promoting apoptosis in vascular smooth muscle cells (VSMCs) and inflammatory cytokine release by macrophages.[11]

Although an increased calcium-phosphate product does tend to promote VC, it is now clear that this process is an active one in which vascular smooth muscle or other mesenchymal elements (such as pericytes)[12] of the vasculature undergo a phenotypic shift, such that their pattern of gene expression and behavior resembles that of osteoblasts.[13] Induced expression of the runt-related transcription factor 2 (*RUNX2*; also called cbf-α-1) plays a key role in this regard, as this transcription factor boosts expression (directly or indirectly) of a number of other proteins found in osteoblasts—including the transcription factors osterix and msh homeobox-2, bone morphogenetic protein-2 and -4 (BMP-2 and BMP-4), alkaline phosphatase, osteopontin, osteocalcin, and matrix Gla protein (MGP)—that enable or regulate extracellular deposition of hydroxyapatite.[14–19] Concurrently, certain proteins that function to inhibit extracellular calcification are downregulated.[20] Extracellular factors believed to play a physiological role in driving this phenotypic transition include: elevated serum phosphate, a major mediator of the VC commonly seen in chronic renal failure, but also linked to increased risk for VC in subjects with normal renal function[21–27]; BMP-2 and BMP-4, members of the transforming growth factor family that contribute importantly to bone formation, but that also can be expressed in the vascular wall[28,29]; advanced glycation end-products (AGEs) that are typically elevated in patients with diabetes[30–33]; other endogenous receptors for AGE ligands such as S100 A12[34–36]; the uremic toxin indoxyl sulfate[37,38]; and certain proinflammatory cytokines commonly expressed in inflamed atheromatous arteries, such as tumor necrosis factor-α and interleukin-6.[39–42] In addition to MGP, endogenous proteins that function to block VC include fetuin, osteopontin, and osteoprotegerin.[43,44] Table 1 lists a number of agents that promote or inhibit the osteoblastic transition of VSMCs, or that work more directly to influence hydroxyapatite deposition.

Table 1. Promoters and Inhibitors of VC

Promoters	Inhibitors
Phosphate	Magnesium
High calcium X phosphate	Adiponectin
BMP-2/-4	Pyrophosphate
AGEs	Matrix G1a Protein
SA10012	Fetuin-A
Indoxyl sulfate	Osteopontin
Tumor necrosis factor-α/interleukin-6	Osteoprotegerin
Leptin	

Abbreviations: AGE, advanced glycation end-product; BMP-2, bone morphogenetic protein-2; BMP-4, bone morphogenetic protein-4; VC, vascular calcification.

A number of recent epidemiological studies link insulin resistance, abdominal obesity, and metabolic syndrome to an increased risk for vascular and valvular calcification.[45–53] Possible mediators of this effect include adiponectin and leptin, which are decreased and increased, respectively, in metabolic syndrome.[54] Low plasma adiponectin predicts progression of coronary calcification, independent of other established risk factors.[55] Moreover, vascular calcification is observed in adiponectin knockout mice.[56] Two studies have reported that adiponectin suppresses calcification in phosphate-treated VSMCs. One of these found that adiponectin decreased RUNX2 expression and the osteoblastic transition,[56] and the other pointed to an anti-apoptotic role of adiponectin in tumor necrosis factor TNF-α–stimulated VSMCs as the basis for its anticalcifying effect.[57] These effects of adiponectin may be contingent on adenosine 5'-monophosphate–activated protein kinase activation.[57] Conversely, there are reports that leptin levels correlate with coronary calcification independently of other pertinent risk factors.[58,59] In atherosclerosis-prone apolipoprotein E–deficient mice, daily leptin injections did not accelerate atherosclerosis per se, but markedly potentiated vascular and valvular calcification.[60] In addition, in vitro, leptin has been shown to promote an osteoblastic phenotype in VSMCs.[61,62] Hence, adiponectin and leptin appear to have a yin-yang relationship with respect to vascular calcification. The possibility that pharmaceutical adenosine 5'-monophosphate–activated protein kinase activators might mimic the protective impact of adiponectin is suggested by a controlled 1-year study in which metformin therapy slowed progression of coronary artery calcification in human immunodeficiency virus—infected patients with metabolic syndrome.[63]

Oxidative Stress

Virtually every physiological agent known to provoke an osteoblastic phenotypic shift in VSMCs has been shown to induce oxidative stress in these cells; moreover, this oxidative

stress appears to drive the phenotypic transition, because measures that prevent or control this stress have been found to block this transition. Exposure of cultured VSMCs to adequate levels of hydrogen peroxide induces RUNX2 and promotes the osteoblastic transition, suggesting that induction of oxidative stress in smooth muscle is both a necessary and sufficient condition for this transition.[17,64] Consistent with this view, markers of oxidative stress are found in the vicinity of calcifying foci in arterial walls and stenotic aortic valves.[65–67] Hydrogen peroxide, rather than other radical intermediates such as superoxide, peroxynitrite, or hydroxyl radical, appears to be the mediating factor in this regard; hence, modulation of the redox status of sulfhydryl groups seems likely to be responsible for the impact of oxidative stress on the phenotypic transition.[66] Increased production (or diminished catabolism) of hydrogen peroxide promotes activation of nuclear factor κB, Akt phosphorylation, and induction of endoplasmic reticulum stress—factors that collaborate to promote the transcription and protein expression of RUNX2, while working in other ways to downregulate factors that oppose ectopic calcification.[17,29] For example, nuclear factor κB suppresses the expression of ankylosis protein homologue, a transmembrane protein that enables extracellular export of pyrophosphate, an antagonist of hydroxyapatite deposition.[20,68]

The primary source of the oxidative stress that drives the osteoblastic transition varies according to the stimulus. Most such stimuli, including BMP-2, AGEs, SA100A12, indoxyl sulfate, inflammatory cytokines, and leptin, have been shown to activate reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complexes in vascular smooth muscle.[29,33–35,38,42,69,70] Adiponectin appears to have the opposite effect.[71] These findings correlate nicely with a recent report that serum levels of free bilirubin—a compound now known to function intracellularly as a potent inhibitor of NADPH oxidase activity[72–75]—correlate inversely with the risk for vascular calcification.[76] Moreover, induction of heme oxygenase-1, an enzyme that generates bilirubin (via biliverdin and biliverdin reductase) from heme, has been shown to block osteoblastic maturation in primary cultured osteoblasts.[77] Conversely, the heme oxygenase-1 activity of calcifying arteries was found to be depressed in rats treated with toxic doses of vitamin D.[78]

Not surprisingly, there are reports that smokers are at greater risk for VC.[79–81] Although there do not appear to be any studies that have examined the impact of smoke extract on osteoblastic transition in VSMCs, the αβ-unsaturated aldehydes and ketones that are prominent constituents of tobacco smoke have been shown to activate NADPH oxidase in a range of tissues by stimulating protein kinase C activity.[82–87] Exposure of rats to cigarette smoke boosts oxidative stress in VSMCs and endothelial cells of their carotid arteries; exposure of carotid arteries to cigarette smoke extract ex vivo has a similar effect.[83] The carotid oxidative stress in rats exposed to cigarette smoke was inhibited ex vivo by diphenyleneiodonium, an inhibitor of NADPH oxidase. Nicotine did not influence arterial oxidative stress in this model. It appears likely that the adverse vascular effects of smoking, including VC, are largely mediated by NADPH oxidase activation.

Concentrations of phosphate comparable to those typically seen in renal failure patients (eg, 2.0–2.6 mM) drive the osteoblastic transition of VSMCs in vitro.[21,22] Intracellular uptake of phosphate via the Pit-1 membrane transporter is necessary for this effect, so an increase in intracellular free phosphate presumably mediates it.[21,88,89] There is recent evidence that exposure of VSMCs to elevated phosphate increases mitochondrial membrane potential while boosting mitochondrial superoxide generation, and that the respiratory chain inhibitor rotenone, but not inhibitors of NADPH oxidase, suppresses the subsequent osteoblastic transition.[90] This finding is at variance with those of a previous study, in which activation of NADPH was found to be at least partially responsible for the phenotypic transition evoked by elevated phosphate.[91] The authors of the more recent study note that they employed primary cells from a bovine artery, whereas the previous study had used an immortalized smooth muscle cell-derived line (A7r5) that was likely to have higher expression of NADPH oxidase and lower mitochondrial activity. Conceivably, activation of NADPH oxidase in the earlier study might have been a downstream consequence of increased mitochondrial superoxide production.

An even more recent study confirms that elevated phosphate exposure promotes oxidant stress in mitochondria, but in this study mitochondrial membrane potential was decreased, and markers of apoptosis were upregulated.[92] Arguably, this may reflect a more intense induction of mitochondrial oxidative stress that damages the mitochondrial inner membrane, leading to a collapse of membrane potential and induction of apoptosis. Indeed, apoptosis of VSMCs is a typical feature of VC, and is suspected to expedite it by promoting nucleation of hydroxyapatite.[13,93]

The possibility that superoxide derived from uncoupled nitric oxide synthase (NOS) contributes to calcification of the aortic valve has been suggested by a study that demonstrated increased superoxide production in stenotic aortic valves

© Postgraduate Medicine, Volume 126, Issue 2, March 2014, ISSN – 0032-5481, e-ISSN – 1941-9260

acquired during surgery or on autopsy.[67] Whereas NADPH oxidase activity was not found to be increased in these valves, inhibitors of NOS suppressed this oxidative stress.

Magnesium

Extracellular magnesium in high physiological concentrations has been shown to block the ability of elevated phosphate to promote an osteoblastic transition in VSMCs; this effect is contingent on intracellular uptake of the magnesium.[94,95] These findings fit nicely with evidence that serum magnesium correlates negatively with the risk for VC and mortality in hemodialysis patients, and a clinical trial observed that supplemental magnesium reduces carotid intima-media thickness in such patients.[96–99] Whether magnesium blocks the impact of elevated phosphate on oxidative stress in VSMCs has not yet been reported, but it can be credibly hypothesized that an increase in intracellular magnesium somehow blunts the pro-oxidative impact of elevated intracellular phosphate on mitochondrial function, possibly via a direct ionic interaction. If this hypothesis is correct, it is conceivable that the protective impact of magnesium on risk for VC is specific to ameliorating the adverse effects of elevated phosphate in this regard, and that VC stemming from other stimuli (such as increased AGEs in patients with diabetes) might not be influenced by magnesium. Further cell culture studies should clarify these issues.

Antioxidant Strategies

The accumulating evidence that hydrogen peroxide is the key driver of osteoblastic transition in VSMCs suggests that antioxidant measures capable of suppressing the production of hydrogen peroxide, hastening its catabolism, or antagonizing its oxidative impact on protein structure could be useful for controlling VC. In light of the ability of bilirubin to inhibit the activity of certain isoforms of NADPH oxidase (the isoform specificity of this effect still awaits clarification), it is interesting to note that the Spirulina chromophore phycocyanobilin (PhyCB), a biliverdin derivative converted by biliverdin reductase activity to the bilirubin homologue phycocyanorubin within cells, has recently been shown to mimic the NADPH oxidase-inhibitor effect of bilirubin, both in vitro and in vivo.[100,101] This phenomenon likely accounts for the versatile anti-inflammatory and antioxidant effects of oral phycocyanin (the Spirulina protein to which PhyCB is covalently attached) observed in a plethora of rodent studies.[101–103] Because there is good reason to suspect that bilirubin may inhibit VC in humans, the impact of oral phycocyanin in rodent models of VC, and of biliverdin and PhyCB in cell culture models of this phenomenon, should be evaluated. It should be noted, however, that PhyCB (or bilirubin) would not be expected to impact the mitochondrial oxidative stress induced directly by increased intracellular phosphate. And whether humans absorb and metabolize PhyCB in a manner comparable to rodents remains to be established.

Intracellular glutathione functions to reverse the oxidations of sulfhydryl groups induced by hydrogen peroxide, and supplementation with the nutraceutical *N*-acetylcysteine can boost intracellular glutathione by increasing the availability of its rate-limiting precursor cysteine.[104,105] This may explain the favorable impact of *N*-acetylcysteine (600 mg twice daily) in a 2-year placebo-controlled trial in hemodialysis patients; the treated patients were 40% less likely to experience a cardiac event during the study (relative risk, 0.60; 95% CI, 0.38–0.95; $P = 0.03$).[106] This study was published over a decade ago, and no attempts to confirm it have appeared yet.

Phase 2 inducers can also boost intracellular glutathione levels (via induction of γ-glutamylcysteine synthase), as well as the activities of various antioxidant enzymes. Lipoic acid has phase 2 inducing potential, and is also suspected of functioning more directly as an antioxidant within mitochondria.[107–111] Lipoic acid has been reported to suppress the osteoblastic transition of VSMCs exposed to elevated phosphate, and when administered orally suppressed VC in mice treated with toxic doses of vitamin D.[92] Conceivably, other phase 2 inducing compounds such as sulforaphane or green tea catechins could provide analogous protection.[112]

Selenium is an essential cofactor for various forms of the enzyme glutathione peroxidase, some of which catabolize hydrogen peroxide. Nutritional selenium availability can be rate-limiting for the expression of selenium-dependent enzymes in regions where soil selenium availability is low, and selenium availability is also suboptimal in many cell culture media.[113,114] This likely explains why preincubation with selenite was found to suppress the osteoblastic transition in VSMCs exposed to hydrogen peroxide in vitro.[64] An improvement in selenium status might be helpful for the control of VC in regions where nutritional selenium intakes tend to be low.

Whether certain antioxidants might have some utility for antagonizing the upregulatory impact of increased intracellular phosphate on mitochondrial superoxide generation remains to be seen. Mitochondrial oxidative stress can exert a feed-forward effect on mitochondrial superoxide production by damaging the respiratory chain of the mitochondrial inner membrane.[115] Indeed, the potent membrane antioxidant astaxanthin has been found to be protective in

ischemia-reperfusion models.[116–118] Whether preincubation with astaxanthin could influence the impact of elevated phosphorus on osteoblastic transition and apoptosis in VSMCs has yet to be determined.

To the extent that uncoupled NOS might contribute to the oxidative stress that drives valvular stenosis, it should be noted that high-dose folate has been shown to promote recoupling of this enzyme, presumably by preventing or reversing the oxidation of the NOS cofactor tetrahydrobiopterin by peroxynitrite-derived oxidants.[119–124] This effect is mediated by high intracellular levels of reduced folate metabolites, which have high and versatile oxidant scavenging activity.[120] This is entirely unrelated to modulation of homocysteine metabolism, and requires higher doses of folic acid than those that optimally suppress homocysteine.[125] Several decades ago, Kurt Oster pioneered the use of high-dose folate (40–80 mg daily) in cardiovascular medicine; his enthusiastic reports were ignored, as they were only anecdotal.[126]

These considerations suggest that certain antioxidants might have the potential to control VC. Nonetheless, these possibilities are currently undocumented and hypothetical from a clinical standpoint and should not be used as a guide to current therapy. At present, evaluation of certain antioxidants in cell culture studies and rodent models of VC would be warranted; clinical trials could then be attempted if these efforts yielded encouraging results. Skepticism regarding the clinical utility of antioxidants is understandable given the null or negative outcomes observed in some major clinical trials involving agents such as α-tocopherol, vitamin C, and β-carotene.[127] However, it should be noted that these compounds would be expected to do little or nothing to block the production or cell signaling impact of hydrogen peroxide, which is why they were not mentioned in the foregoing discussion.

Minimizing Phosphate Absorption

A range of additional measures may be helpful in the prevention of VC. Magnesium supplementation has been cited above; this might work not only via a direct effect of elevated magnesium on VSMCs, but also by promoting precipitation of dietary phosphate in the gastrointestinal tract, opposing its absorption.[128,129] Moreover, a number of practical measures have the potential to moderate serum phosphate levels by minimizing the amount of phosphate absorbed from the diet: choosing a plant-based diet, in which a high proportion of the phosphate is tied up in poorly absorbable phytates; avoiding soft drinks and processed foods that contain phosphate additives; employing phosphate-binding drugs such as sevelamer or lanthanum carbonate, as is commonly done in treatment of chronic renal disease; magnesium supplementation; and treatment with sustained-release niacin, which appears to decrease gastrointestinal expression of a prominent phosphate transport protein.[128–140]

Vitamin K and Vitamin D

Vitamin K can favorably influence VC, as posttranslational γ-carboxylation of MGP, catalyzed by a vitamin K–dependent enzyme, is crucial to MGP's ability to antagonize VC.[141,142] This presumably explains why use of the vitamin K–antagonist drug warfarin is associated with increased risk for VC.[143] How properly carboxylated MGP functions in this regard is still unclear; it can bind to and antagonize the activity of the BMP proteins, which may play a prominent role in VC, but additional mechanisms are being explored.[144–146] Serum levels of dephospho-undercarboxylated MGP have been found to correlate directly with VC, and these levels can be decreased dose-dependently by effective vitamin K supplementation.[147–150] The menaquinone form of vitamin K (vitamin K2) produced by certain bacteria appears to be more effective for promoting carboxylation of MGP than the phylloquinone form found in green leafy vegetables and algae, presumably because the former is more effectively transported to peripheral tissues (as opposed to the liver).[151] In epidemiological studies, increased intakes of menaquinone, but not phylloquinone, have been linked to a lower risk for coronary calcification and coronary heart disease.[152–154] But significant amounts of vitamin K2 are found only in certain fermented soy products (notably nattō) and certain cheeses. Hence, aside from aficionados of nattō (which is popular in eastern Japan),[155] the vitamin K activity of most people eating natural diets is suboptimal from the standpoint of MGP γ-carboxylation and, presumably, from the standpoint of VC. Supplemental intakes ≥ 200 μg menaquinone-7 daily appear to achieve near maximal γ-carboxylation of MGP and other vitamin K–dependent proteins.[151] Although the highly favorable impact of vitamin K2 supplementation on bone strength and fracture risk has been documented (reflecting vitamin K's role in γ-carboxylation of osteocalcin),[156] its impact on risk for VC should be addressed in future studies. Importantly, vitamin K status tends to be poor in hemodialysis patients, who of course are at high risk for VC.[157,158]

Poor vitamin D status (low serum 25-hydroxyvitamin D) has been associated clinically with increased VC, whether or not renal function is impaired, and such calcification is seen in mice fed a vitamin D–deficient diet.[159–163] On the other hand, toxic intakes of vitamin D promote VC by increasing serum

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levels of unbound calcitriol, which functions to boost absorption of both calcium and phosphate.[164,165] Why moderate vitamin D activity is protective with respect to VC remains unexplained. Although the cardiovascular protection afforded by good vitamin D status is likely mediated in large part by downregulation of parathyroid hormone (PTH) levels,[166,167] PTH has not been found to promote osteoblastic transition in smooth muscle cell cultures; indeed, stimulation of the membrane PTH receptor on VSMCs has been reported to suppress this transition.[168,169] On the other hand, continuous intravenous infusion of PTH was found to promote VC and RUNX2 expression in parathyroidectomized, 5/6 nephrectomized rats, so it is conceivable that PTH promotes VC through some indirect mechanism.[170] Another possibility is that vitamin D directly influences vascular smooth muscle function, as the 1-α-hydroxylase activity required for conversion of 25-hydroxyvitamin D to the active hormone calcitriol has been reported to be present in cultured VSMCs.[171] Although a recent report indicates that calcitriol and other vitamin D receptor agonists can inhibit the osteoblastic transition in VSMCs in vitro,[172] previous reports indicated either no effect or an adverse effect in this regard.[173–175] It is not yet clear whether autocrine production of calcitriol in vascular smooth muscle is high enough to be of physiological significance in vivo. Vitamin D supplementation, in daily doses within the range of 2000 to 10 000 International Units, appears to be an appropriate and safe strategy for ensuring replete vitamin D status.[176]

In renal failure patients, the capacity to convert 25-hydroxyvitamin D to calcitriol is typically impaired (though usually not absent),[177,178] so treatment with vitamin D agonist drugs, not requiring 1-α-hydroxylase activation for activity, is commonly employed. These agents are a mixed blessing, as they have the potential to promote VC by expedited absorption of both calcium and phosphate; hence, relatively low doses of these agents may provide the greatest net benefit for vascular health. Paricalcitol, which appears less likely than certain other vitamin D agonist drugs to promote excessive calcium/phosphate absorption, may have a more favorable effect than calcitriol on phosphate-stimulated osteoblastic transition in vitro.[179,180]

Etidronate

As noted, one strategy that VSMCs employ to prevent ectopic calcification is to generate pyrophosphate within the extracellular matrix, either by ectonucleotide pyrophosphatase activity or by exporting pyrophosphate via the ankylosis protein homologue transporter.[68] Pyrophosphate not only antagonizes hydroxyapatite deposition by a direct biophysical effect, but also acts on VSMCs to prevent an osteoblastic phenotypic shift.[181] (Conversely, alkaline phosphatase degrades the extracellular pyrophosphate pool, thus accounting for its key role in bone formation and VC.) The bisphosphonate drug etidronate is a highly stable analogue of pyrophosphate that shares its ability to block hydroxyapatite deposition. Two small controlled clinical trials have evaluated the impact of intermittent etidronate on VC in hemodialysis patients with favorable results. A regimen of 200 mg/day for 14 days every 3 months led to a reduction in coronary artery calcification that was significant relative to controls; bone density was not changed.[182] (A similar regimen in patients with type 2 diabetes was reported to decrease carotid intima-media thickness).[183] Another controlled study evaluated 200 mg etidronate given every day of dialysis; the aortic calcification area failed to progress under this treatment, whereas it continued to increase in the control group; again, bone density was not influenced in the treated group.[184] In a rat model of renal failure and VC, etidronate administration likewise inhibited aortic calcification without modifying bone density.[185] These results suggest that intermittent etidronate merits more extensive evaluation in groups at high risk for VC.

Conclusion: Practical Strategies for Avoiding VC

There is considerable evidence that induced oxidative stress—stemming from NADPH oxidase, mitochondria, and possibly uncoupled NOS—drives the osteoblastic phenotypic transition of VSMCs that mediates vascular and valvular calcification. Therefore, drugs and nutraceuticals that either inhibit the production of hydrogen peroxide or oppose its impact on cellular signaling in VSMCs may have the potential to prevent VCs; they may include the bilirubin analogue phycocyanobilin, as well as *N*-acetylcysteine, lipoic acid, high-dose folate, and selenium. Elevated phosphate levels trigger mitochondrial oxidative stress in VSMCs. Measures that decrease vascular exposure to phosphate have been shown to be useful for controlling VC, especially in those with compromised renal function; a plant-based diet, avoidance of phosphate additives, pharmaceutical phosphate binders (sevelamer, lanthanum carbonate), supplemental magnesium, and niacin may be useful in this regard. Magnesium acts on VSMCs directly to counteract the impact of phosphate on osteoblastic transition. Optimizing vitamin K status, preferably via supplementation with menaquinone-7, helps to prevent VC by promoting sufficient γ-carboxylation of the calcification

Table 2. Strategies for Inhibiting VC

Reducing phosphate absorption—for moderating serum phosphate
Plant-based diet
Avoidance of phosphate additives
Pharmaceutical phosphate binders or magnesium
Niacin
Antioxidants—potentially useful for inhibiting osteoblastic transition of VSMCs
Phycocyanobilin, *N*-acetylcysteine, lipoic acid
Magnesium—suppresses phosphate-driven osteoblastic transition of VSMCs
Vitamin K—promotes γ-carboxylation of MGP
Vitamin D—protective when adequate but not toxic; mechanism unclear
Etidronate—pharmaceutical pyrophosphate mimic
Correct/control metabolic syndrome and diabetes
Smoking cessation

Abbreviations: MGP, matrix Gla protein; VC, vascular calcification; VSMC, vascular smooth muscle cell.

antagonist MGP. Maintaining replete vitamin D status may also provide protection from VC, for reasons that are unclear, whereas vitamin D toxicity promotes VC by boosting calcium and phosphate absorption inappropriately. Prevention or control of metabolic syndrome and diabetes with prudent lifestyle measures, as well as the avoidance of tobacco, also appears likely to diminish the risk for VC. Etidronate may have potential for inhibiting VC by mimicking the protective impact of pyrophosphate in this regard. These strategies are summarized in Table 2.

Conflict of Interest Statement

Mark F. McCarty, BA, is a coinventor of the nutraceutical use of phycocyanobilin oligopeptides extracted from Spirulina, for which a patent is pending. Mr McCarty also serves on the advisory committee and is a stockholder of NutriGuard Research, Inc.; he is a consultant for Whitaker Wellness Institute and Oasis of Hope Hospital; and he is a research fellow for Catalytic Longevity. James J. DiNicolantonio, PharmD, has no conflicts of interest to declare.

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