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Would acetazolamide inhibit progression of atheromatous calcification?

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BRIEF REPORT

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ABSTRACT

Background

Vascular calcification is a recognised source of morbidity among mid-age and elderly subjects. Its development follows classical mineralisation pathways, inhibited by acidosis.

Aims

To examine the argument for using acetazolamide to retard vascular calcification.

Results

The final mechanism of tissue mineralization involves three processes, all of which are highly pH dependent. Calcium interacts with phosphate in its trivalent form, but this step is inhibited by pyrophosphate, the substrate for alkaline phosphatase. Separately, matrix vesicles create nucleation sites and indirectly disrupt vascular smooth muscle cells. Metabolic acidosis acts at every point to delay mineralization. The diuretic acetazolamide creates a sustained mild acidosis and phosphate loss and, though usually ineffective in the experimental model, has been used with success in certain clinical conditions.

Conclusion

We suggest that acetazolamide, well studied and tolerated, could be trialled in selected subjects to offset the progression of vascular calcification, through its dual action of lowering tissue pH and phosphate concentration.

Key words

Vascular calcification, final mineralization process, pH effect, acetazolamide

Implications for Practice:

1. What is known about this subject?

Vascular calcification is a recognised source of morbidity among mid-age and elderly subjects. Its development follows classical mineralisation pathways, inhibited by acidosis.

2. What new information is offered in this report?

The diuretic acetazolamide creates a sustained mild acidosis and phosphate loss but is ineffective in the experimental model which generally involves induced renal failure. It should be able to inhibit vascular mineralisation in the absence of renal failure.

3. What are the implications for research, policy, or practice?

Acetazolamide could be trialled in selected subjects to offset calcification, through its dual action of lowering tissue pH and phosphate concentration.

Background

The many aspects of vascular calcification have been the subject of a great deal of investigative work over the last two decades. The sub-intimal and medial forms both involve the layer of smooth muscle cells (VSMC), with the former following atheromatous inflammatory damage, and the latter, Monckeberg's sclerosis, occurring with age. The arterial wall consists of the thin endothelial layer, lying on the elastic tunica intima, supported by the tunica media, mainly VSMC. The latter provide strength and responsiveness but they also secrete the collagen and elastin fibres and proteoglycans which contribute to the vessel's structure. These cells are analogous to fibroblasts, osteoblasts or chondrocytes at their respective sites.[1] When atheroma disrupts the sub-intimal layer of VSMC, the foreign material, especially LDL, attracts macrophages,

particularly those with an inflammatory response.[2,3] Simply with age, more deeply in the media, the differentiation of VSMC into fibroblasts leads to stiffening of the wall.[4] In either case, the process is not benign with the inelasticity and irregular lumen increasing shear stress and turbulence in the blood flow.[5,6] The association between vascular calcification in the coronary arteries and plaque stabilisation or destabilisation is unclear[7,8] though it is generally agreed that there is increased risk of thrombosis. However, at a given site, there is no clear link between the two.[9]

The prevalence of the condition among adults is remarkably high. Based on samples of femoral-popliteal arteries from a large number of transplant donors, about 50 per cent of cases showed calcification[10] and similar results were found in a study using non-invasive techniques[11] where it was a common finding (c.70 per cent) among elderly subjects but was also evident in some young adults. Obesity was a significant risk factor, dominating the effect of tobacco.[10]

It is abundantly evident that the calcification, when it occurs, is a complex process mimicking many aspects of normal bone formation;[12,13] however, that in itself suggests targets to inhibit progression.

A surprising finding has been the observation that Statin use is apparently associated with increased vascular calcification as shown by imaging.[14,15] It is unclear if this is a direct effect or simply follows inactivation and scarring down of atheromatous plaques.

Discussion

The discussion and suggestion that follows refer to the mineralisation process in subjects with a normal or near normal metabolic state, in particular excluding the case of renal failure which is, however, the usual model in non-clinical studies. Nor have we considered the upstream subtleties of bone formation modulated by, for example, FGF23 and alpha-KLOTHO, Vit D metabolite receptors, osteoclast control factors (RANK and RANK Ligand), Osteoprotegerin or Sclerostin and possibly other glycoproteins. We confine the argument to actual mineralisation in its final stages, these being sensitive to pH change.

Mechanisms of vascular calcification

In normal bone mineralisation and vascular calcification, the final product is hydroxy-apatite but this stable salt is the end product of a series of transformations starting with octocalcium phosphate.[16,17] The two determinants of octocalcium phosphate formation in an aqueous medium are the concentrations of calcium and trivalent phosphate ions. The phosphate species in the ECF are made up of mono-, di-, and trivalent phosphate, and it is the strongly negative trivalent phosphate ion that interacts with the positively charged divalent calcium. Of the total plasma phosphate concentration, only a small fraction exists as trivalent phosphate and this is significantly pH sensitive. Even in mild acidosis, e.g. pH 7.3, there is ultimately about a 20 per cent decrease in the prevailing concentration of trivalent phosphate and so the rate of complexation with calcium is correspondingly slower.

This effect of pH shift on trivalent phosphate is only one of three closely matching mechanisms which explain the mineralisation failure of acidosis. The product of cellular reactions involving high energy phosphate bonds is pyrophosphate (POP). The classic source of POP is deactivation of ATP but most molecules in a chain of reactions require a high energy phosphate bond. This molecule is a strong inhibitor of mineralisation[18] and its breakdown product, the phosphate ion, means that it has a dual role in any shift between promotion or prevention of mineralisation. More POP means less local phosphate. The enzyme which degrades POP is alkaline phosphatase,[19] so acidosis would mean the concentration of POP would be relatively higher,[20] tending to slow mineralisation. Accordingly, a fall in plasma pH inhibits calcification directly, through reduction of local phosphate concentration, sourced from POP, and further reduction of the trivalent moiety; and indirectly by reducing hydrolysis of inhibitory POP.

Although the inhibitory action of Statins blocks a series of downstream reactions, which would otherwise release POP molecules, these are confined to the hepatocyte, so cannot be considered relevant to this discussion.

Matrix vesicles were described many years ago, first in calcifying cartilage[21] then in mouse calvaria.[22] They bud off as portions of the cell membrane, carrying alkaline phosphatase, and actively accumulate calcium to begin the process of mineralisation in the intercellular space, making it independent of cellular activity. They have a definite role in vascular calcification.[23,24] Their uptake of calcium is strongly reduced with lower pH thus introducing a further point for inhibition.[25,26]

The VSMC may pick up some of the mineral particles by pinocytosis, disrupting their structure.[8,26,27] On the other hand, there is a clear picture of VSMC differentiating into fibroblast or osteoblast cell types, with the appearance of

all the known signalling molecules for mineralisation and its production as discussed here. This would match the effect seen with ageing. Ultrastructural studies suggest that inside the cell, long crystalline hydroxy-apatite structures appear within an amorphous sheath, along with known trophic factors for bone formation.[26]

Calcification may additionally be promoted by induction of mineralisation pathways in the presence of an elevated plasma phosphate but this probably does not apply in normophosphataemia:[28-30] it would require a substantial degree of renal failure.

There are then two forms of calcification at work: one, somewhat haphazard and destructive to the cell architecture; the other a cellular change with the features of organised mineralisation.[6,26]

A role for induced acidosis

Given the requirement for a physiologic pH for mineralisation to proceed, and the evidence for inhibition by metabolic acidosis, we can consider the possibility of offsetting the final stage of vascular calcification by inducing a mild acidosis.

This is easily achieved with the diuretic acetazolamide, a carbonic anhydrase inhibitor, the target enzyme being found particularly in the kidney, brain, and stomach. It was initially of minor use, but was found to have some anti-epileptic properties and also to reduce sleep apnoea at high altitude.[31] Many other studies have shown that it increases cerebral blood flow; though it is unclear how that affects the neurone.[32] It is well tolerated and with usual dosing has no significant side-effects.[33]

As a renal carbonic anhydrase inhibitor, it prevents hydrolysis of bicarbonate in the proximal tubule, increasing the negative luminal potential, so reducing sodium reabsorption and associated movement of water. The loss of bicarbonate from plasma shifts the equilibrium towards a sustained acidosis with a fall in plasma bicarbonate of about 4mmol/L[33] corresponding to a change in blood pH from ~7.4 to ~7.3 for about 12 hours. Phosphate, reabsorbed proximally by Na-P co-transporters, also pH sensitive,[34,35] is washed downstream with a decrease in plasma level.

Acetazolamide therefore creates a reduction both in pH and plasma phosphate. Both effects, but the pH fall especially, would be central in inhibiting mineralisation. There are reports of its successful use even in the hyperphosphataemic state, e.g. tumorous calcinosis.[36-38] A comprehensive review of the pathophysiology of vascular calcification using laboratory models generally with induced severe renal impairment, supported the idea of prevention by acidosis, but understandably found acetazolamide rather ineffective in this setting.[39,40]

Consideration of the use of acetazolamide to reduce progression of vascular calcification

Acting at the kidney level, the drug would obviously be ineffective among patients with chronic renal failure and, promoting acidosis, unsuitable in Insulin Dependent Diabetes. Thiazide diuretics, which produce potassium loss and alkalosis, would, if in place, need to be substituted with the acetazolamide. With these provisos, we argue that acetazolamide may offer a practical way to reduce progression of vascular calcification, a recognised risk-factor for tissue ischaemia and morbidity among the elderly population.

Conclusion

We suggest there exists a case for investigating its use. A population based study would be one approach, using ultrasonography,[11] in subjects across an elderly age group with random assignment to treat with a sub-maximal dose (125mg nocte or more if tolerated) of acetazolamide over a year. Alternatively, within a clinic, whether for coronary artery or peripheral vascular disease, using the same dose, a more formal open trial could be undertaken with CT, followed by a 12 months review of a chosen site. A fall in plasma bicarbonate of ~4mmol/L would be required. The subjects would almost certainly be prescribed a Statin, and this would have to be standardised, particularly to achieve a targeted LDL response. Either approach might lead to interest in or rejection of the idea a subsequent formal double blind RCT.

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CONFLICTS OF INTEREST

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