Resolvin E1 for reducing vascular calcification

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Vascular calcification - the deposition of excess mineral within the vessel wall - is now recognized as a complex, actively controlled process, principally mediated at the level of the vascular smooth muscle cell (VSMC). The extent of calcium deposition within the vessel wall can be used to predict the risk of adverse cardiac events (ACEs) (1).

In the absence of vascular pathology, VSMCs exist in a quiescent state characterized by a set of contractile proteins such as CNN1 (calponin 1) and ACTA2 (alpha smooth muscle actin) which allow VSMCs to carry out their primary function, namely to provide structural support and to mediate vasoreactivity. In response to vascular injury/pathology, VSMCs can undergo a phenotypic transition towards a more synthetic phenotype. This change is characterized by downregulation of contractile proteins, increased proliferation and migration and enhanced extracellular matrix production. It is now widely thought that in disease, VSMCs form a heterogenous population with some VSMCs gaining an osteochondrogenic phenotype associated with osteogenic markers such as runt-related transcription factor 2 (RUNX2), type 1 collagen (Col1A1) and enhanced response to bone morphogenetic protein-2 (BMP-2) (2). The accumulative effect of this phenotypic switch is a cell type much more prone to accumulating calcium deposits within the vessel wall; however, the molecular pathways that promote this event are still not well defined.

In the study by Carracedo and colleagues (3) recently published in *Cardiovascular Research*, the authors investigated the role of the ChemR23 receptor in calcification of epigastric arteries derived from kidney transplant patients. In chronic kidney disease, vascular calcification is an independent predictor of cardiovascular morbidity and mortality and the primary inducer of vascular calcification in these patients is thought to be hyperphosphatemia (4). ChemR23 is a G-protein coupled receptor expressed on multiple cell types including VSMCs (5), and is upregulated during bone development. Chemerin was the first ChemR23 ligand discovered and induces pro-inflammatory properties via ChemR23 agonism (6). Subsequently, resolvin E1 (RvE1), an inflammatory resolving lipid mediator derived from the omega-3 fatty acid eicosapentaenoic acid (EPA), was also described in the literature as being a ligand for ChemR23 (7); however a direct interaction between RvE1 and ChemR23 is somewhat

53 controversial with other receptors such as the leukotriene B4 receptor BLT1 also a candidate
54 for mediating the effects of RvE1 (8). In contrast to chemerin, the RvE1 interaction with
55 ChemR23 has been reported to produce anti-inflammatory activity. RvE1 and other omega-3
56 derived specialized proresolvin mediators (SPM) have shown promise in resolving
57 inflammation in several diseases in humans (9). Moreover, data from animal models indicates
58 that administration of RvE1 results in reduced atherosclerosis (10), and that ERV1/ChemR23
59 signaling protects against atherosclerosis (11). However, the role of ChemR23 and RvE1 in
60 VSMC calcification and osteogenesis has been unexplored until now.
61 Gene expression analysis of patient arteries revealed ChemR23 to be an independent
62 predictor of Col1A1. To obtain more insight into the effect of ChemR23 on VSMC phenotype,
63 the authors next cultured VSMCs from ChemR23$^{-/-}$ and wild-type mice. Under cell culture
64 conditions, VSMCs spontaneously de-differentiate yet this process was greatly attenuated in
65 ChemR23$^{-/-}$ VSMCs. Furthermore, in response to elevated phosphate levels, calcification of
66 cells was also attenuated in ChemR23-deficient VSMCs, as was activation of BMP-2 signaling,
67 a known inducer of calcification in VSMCs under high phosphate conditions (2). Concomitant
68 with reduced calcification was a reduction in pro-calcific RUNX2 and an increase in anti-calcific
69 osteoprotegerin (OPG). To test the hypothesis *in vivo*, mice were injected with vitamin D3,
70 which mimics phosphate induced calcification. ChemR23$^{-/-}$ mice had reduced calcification in
71 the medial layer of the aortic root and carotid artery. These were accompanied by biochemical
72 changes indicative of a less calcific, more contractile VSMC phenotype, despite ChemR23$^{-/-}$
73 mice displaying more pronounced hyperphosphatemia. This strengthens the view that the loss
74 of ChemR23 is acting directly via VSMCs and not due to systemic metabolic changes, thus
75 supporting the *in vitro* observations. RvE1 treated wild-type VSMCs showed reduced
76 calcification and BMP-2 expression, effects not observed in ChemR23$^{-/-}$ VSMCs. The
77 reduction in calcification was not associated with other changes in VSMC phenotype indicating
78 RvE1 has calcification specific effects mediated by ChemR23. Finally, the authors introduced
79 the *C. elegans* Fat 1 transgene into wild-type and ChemR23$^{-/-}$ mice. Fat 1 encodes a fatty acid
80 desaturase that converts omega-6 to omega-3 fatty acids. The rationale being that enrichment

of EPA results in greater production of RvE1 as previously observed (12). In support of RvE1 induced inhibition of ChemR23 mediated vascular calcification, the authors noted that ChemR23 dependent effects were attenuated in the presence of increased endogenous omega-3 production, potentially due to RvE1 inhibitory action (3).

Derivatives of the omega-3 fatty acids EPA and docosahexaenoic acid (DHA) are thought to possess anti-inflammatory properties, acting to oppose the pro-inflammatory actions of cyclo- and lipoxygenase arachidonic acid products. The current work indicates that EPA derived RvE1 may also help in preventing the adverse phenotypic changes in VSMCs, such as accumulation of calcium, that run in parallel with vascular inflammation across a range of vascular pathologies. A multitude of studies looking at the cardioprotective effects of omega-3 supplementation have failed to reach a consensus on whether there is a beneficial effect on CVD morbidity or mortality. Recently, attention has focused on the EPA content in omega-3 formulations. A relatively low daily EPA dose (469 mg; 1-gram total omega-3) formulation proved ineffective in reducing ACEs in diabetic patients (13). A different trial involving more than 8000 participants across 11 countries employed a total daily dose of 4 g of a purified EPA ethyl ester formulation in patients at risk of CVD and with elevated triglycerides. Despite the concurrent use of statins, patients taking the EPA treatment had a 25% reduction in risk of ischemic events, including cardiovascular death, compared to those who received placebo (14). A similar trial (STRENGTH - Statin Residual Risk Reduction With Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia; ClinicalTrials.gov number, NCT02104817) is currently looking at the use of an EPA enriched omega-3 formulation (Epanova) in modifying ACEs in approximately 13000 statin treated patients. A positive outcome from both these trials would increase the therapeutic focus onto EPA and its derivatives.

The understanding of endogenous SPM production as well as blood and tissue concentrations is an emerging area of research and the modulating role of these compounds in human disease is still unknown. Results between human subject studies to date are mixed regarding detection of circulating SPMs (15) and it is currently unclear what the dose-relationship is to

dietary intake of EPA. Translating *in vivo* based studies to human disease will require sensitive mass spectrometry profiling of SPMs in biological fluids from patient and control subjects. This could determine if circulating SPMs are biomarkers for CVD and may offer insight into the relationship between omega-3 intake and cardiovascular protection. Such personalized information based on SPM production could enable clinicians to better tailor omega-3/EPA doses in future trials and may shed light on why some patients show a therapeutic response to omega-3 intake and others do not. A key future goal would also be to determine if a failure to resolve inflammation is related to depleted local SPM levels and if administered EPA/DHA could reverse this trend. Furthermore, the identification of ligands and receptors such as RvE1 and ChemR23 respectively could allow the use of more refined lipid preparations and also inform pharmacological approaches to design higher potency SPM analogues with a favorable anti-inflammatory/VSMC preserving profile.

In summary, the current work highlights a novel role for the ChemR23 receptor in promoting VSMC differentiation towards an osteoblastic, synthetic phenotype and highlights RvE1 as an inhibitory ligand against ChemR23 mediated VSMC calcification, a hallmark feature of human vascular pathology. Given the current lack of treatments for reversing or halting VSMC calcification, the discovery that RvE1 as an inhibitor of this process is an important development and may contribute to the cardiovascular benefits of EPA.

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Conflict of Interest

None declared.

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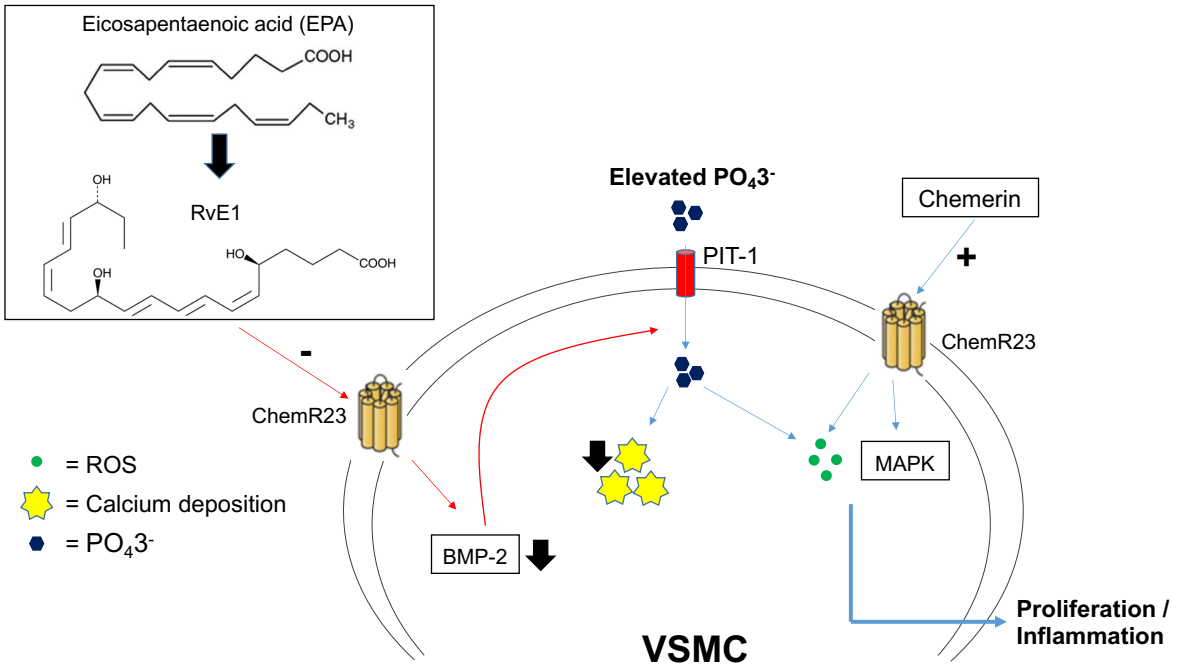

Figure 1. ChemR23 as a mediator of VSMC dysfunction. RvE1 is derived from the Omega-3 fatty acid, EPA (box). By acting as an inhibitory ligand on ChemR23, RvE1 causes a reduction in BMP-2 gene expression. BMP-2 promotes phosphate uptake via sodium-dependent phosphate cotransporter PIT-1, and through this mechanism, enhances VSMC calcification. Therefore, the reduction in BMP-2 expression observed after RvE1 treatment in ChemR23$^{+/+}$ cells likely results in reduced calcification due to reducing the accumulation of intracellular phosphate. This effect was mediated by ChemR23 since ChemR23$^{-/-}$ VSMCs did not show changes in calcification following RvE1 treatment. This study also reveals ChemR23 to be a positive modulator of VSMC proliferation/differentiation. A possible candidate for ChemR23 activation is chemerin, a ChemR23 agonist. Activation of ChemR23 can induce aberrant VSMC phenotypic changes through enhancement of reactive oxygen species (ROS) and MAPK signaling. BLACK arrows show observed phenotypic changes and RED arrows show pathways inhibited after RvE1 treatment under elevated phosphate (PO_4^{3-}) conditions.