The role of OPG/RANKL in the pathogenesis of diabetic cardiovascular disease

Hannah Forde^a, Colin Davenport^a, Emma Harper^b, Phil Cummins^{b,c} and Diarmuid Smith^{a,c}

Cardiovascular (CV) disease is the leading cause of mortality in patients with type 2 diabetes mellitus. A major factor in the pathogenesis of CV disease is vascular calcification (VC), which is accelerated in type 2 diabetes mellitus. Calcification of the vessel wall contributes to vascular stiffness and left ventricular hypertrophy whereas intimal calcification may predispose to plaque rupture and CV death. The pathogenesis of VC is complex but appears to be regulated by the osteoprotegerin (OPG)/receptor activator of nuclear factor- κ B ligand (RANKL) signaling pathway, which is involved in bone remodeling. Within the bone, OPG prevents RANKL from binding to receptor activator of nuclear factor- κ B and inhibiting bone resorption. Outside of the bone, the clinical significance of OPG blocking RANKL is not well understood, but OPG knockout mice that lack OPG develop early and severe VC. This minireview outlines some of the research on OPG/RANKL

in the pathogenesis of VC and discusses potential therapies, which may reduce VC and CV burden in humans. *Cardiovasc Endocrinol Metab* 7:28–33 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

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^aAcademic Department of Diabetes Beaumont Hospital, Royal College of Surgeons in Ireland, ^bDepartment of Vascular Biology, Dublin City University and ^cThe 3U Diabetes Group Ireland, Dublin, Ireland

Correspondence to Diarmuid Smith, MBBCh, BAO, MD, FRCPI, Diabetes Day Centre, Beaumont Hospital, Beaumont Road, Dublin 9, Ireland Tel/fax: + 353 1857 2979; e-mail: diarmuidsmith@beaumont.ie

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In the developed world, cardiovascular disease (CVD), including coronary heart disease and stroke, remains the commonest cause of death, and in diabetes, ~65% of all deaths in patients with type 2 diabetes mellitus (T2DM) occur because of CVD [1]. Our research group has been interested in the role of vascular calcification (VC) in the pathogenesis of CVD in diabetes.

As recently as 25 years ago, the widespread view of calcification within the vasculature was of a passive process of calcium deposition in the vessel walls that was essentially benign in terms of its effects on CVD [2]. This viewpoint has changed in recent years with the recognition that VC is an active process involving the transformation of certain vascular cells within the vasculature into osteoblastic cells similar to those involved in bone formation [3–6] and is a pathophysiological process that affects multiple aspects of the vascular tree, in particular the intimal and medial aspects of the arterial wall [7,8]. VC exerts numerous detrimental effects on the vasculature, contributing to increased arterial stiffness, widened pulse pressure, increased rates of left ventricular hypertrophy, and increased risk of coronary artery dissection after angioplasty [9], and studies have specifically

linked VC to cardiac death, as coronary artery calcification (CAC) in coronary artery disease (CAD) has been shown to be significantly associated with mortality [10]. Moreover, intimal VC has been claimed to be an independent risk factor for cardiovascular (CV) death [11]. As CVD is the major cause of mortality among diabetes patients [12], it is unsurprising that VC is a pathological condition frequently accelerated in subjects with T2DM [13,14].

Despite the recognition of a clear association between VC burden and rates of CV morbidity and mortality, the cellular mechanisms that promote and inhibit VC remain unclear, and clinical interventions aimed at inhibiting or reversing VC have produced, for the most part, disappointing results [15].

The pathogenesis of VC appears to be controlled by the osteoprotegerin (OPG)/receptor activator of nuclear factor- κ B ligand (RANKL)/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling pathway, which is involved in bone remodeling. OPG is a soluble member of the tumor necrosis receptor superfamily, which exerts its biological functions by acting as a decoy receptor for either RANKL or TRAIL. Within the skeletal system, the interaction and clinical relevance of OPG and RANKL are well-described. In essence, OPG prevents RANKL from binding to the receptor activator of nuclear factor- κ B receptor on osteoclast precursor cells,

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thereby preventing the maturation of osteoclasts, with the result being a net gain in bone mineral density [16]. Outside the skeletal system, the clinical significance of OPG blocking RANKL and/or TRAIL signaling is not well understood, but OPG knockout mice that lack OPG develop early and severe VC [17]. OPG has been identified as existing within the vasculature in significant amounts, and a study by Morony *et al.* [18] reported that the administration of OPG to atherogenic mice led to a reduction in calcification burden, whereas in a separate study, the coadministration of OPG with vitamin D prevented VC in mice [19].

RANKL, however, actively promotes the calcification process in vascular cells through an ability to act as an inducer of osteoblastic activity [20]. RANKL, when secreted by ECs, can bind to the receptor activator of nuclear factor- κ B receptor to promote pathological differentiation of healthy VSMCs into calcified VSMCs with an osteoblastic phenotype [21]. In this respect, RANKL is upregulated in calcified VSMCs [22] and has been shown to exert its procalcification actions through activation of the NF- κ B pathway [20]. Thus, when serum RANKL levels are high, an acceleration of this differentiation process occurs, resulting in an increased mineral deposition within the medial arterial wall [21]. Likewise, OPG, which is also expressed in the vasculature by ECs and SMCs, acts as a soluble decoy receptor for RANKL. OPG can bind to and neutralize RANKL to ameliorate the VC process [23], thereby exerting an anticalcific effect within the vasculature (Fig. 1). To further support the proposed OPG/RANKL relationship, isolated murine OPG^{-/-}ApoE^{-/-} VSMCs developed increased calcification and exerted an upregulation of osteochondrogenic genes following RANKL treatment, whereas OPG^{+/+}ApoE^{-/-} VSMCs exhibited no such response. This basic model highlights the protective effect of OPG on VC through the modulation of RANKLs procalcific effects [26]. Thus, both RANKL and OPG appear to exert the 'opposite' effects during VC to those typically exerted by either of these ligands during bone remodeling [27], an apparent pathophysiological 'paradox'. A third regulatory protein, TRAIL, has been shown to putatively interact with OPG and RANKL during modulation of the VC process [28], and an emerging hypothesis within the VC field has proposed a vasoprotective role for TRAIL, possibly through pleiotropic effects on vascular gene expression and/or an ability to mediate RANKL signaling. Systemic delivery (both single/repeated injection) of recombinant TRAIL to ApoE-null diabetic mice demonstrated antiatherosclerotic activity [29], and it has been reported that TRAIL has the ability to counteract RANKL's procalcific signals in both cell culture [30] and murine models [31]. A further discussion on TRAIL is beyond the remit of this article, but I would refer readers to a recent review article on TRAIL [32]. In contrast to these observations however, it

has also been claimed that neither OPG, RANKL, nor TRAIL has any effect on VSMC calcification *in vitro* [23] and that RANKL has no direct effect on VSMCs [33]. The discrepancy in results is likely because of the methodological differences in studies, with some studies using phosphate-containing procalcific growth media, which can enhance calcification, and phosphate itself, which can induce osteoblastic activity. Furthermore, endogenous OPG secretion, primarily by VSMCs [33], leading to RANKL neutralization, is often overlooked, whereas static VSMC monocultures lack endothelial paracrine signaling inputs and shear-mediated conditioning effects that are undoubtedly present in the corresponding in-vivo environment [34,35].

In clinical studies, high levels of plasma OPG have been shown to positively predict CVD morbidity and mortality [36], whereas circulating OPG is increased in patient groups with high levels of arterial calcification [37]. In the Dallas Heart Study, it was observed that CAC and aortic plaque volume was positively associated with circulating OPG in an unselected population, thereby indicating its possible use as a biomarker for atherosclerosis [38]. Moreover, high levels of OPG have been positively correlated with CAD [39] and peripheral vascular disease [40], whereas Omland *et al.* [41] have highlighted its potential use as a predictor of heart failure and long-term mortality in patients who experience acute coronary syndromes. Higgins *et al.* [42] have demonstrated that both tissue and serum OPG are strongly and inversely associated with calcification in human carotid atherosclerosis. Elevated serum OPG has also been linked to T2DM. In murine models for example, it has been shown that OPG levels increase shortly after induction of diabetes [29], with a similar trend noted in clinical studies. Many studies have significantly correlated serum OPG elevation with worsening CV burden in T2DM, including CAC [43], carotid intimal-medial thickness [44], hypertension [45] coronary/peripheral arterial disease [46], metabolic syndrome, and microvascular complications [47]. Elevated OPG has also been shown to invariably predict coronary artery VC progression in diabetics, and furthermore can be used to predict future CV events [48]. Finally, a 2012 study into advanced carotid atherosclerosis illustrated that a history of diabetes and CAD could independently predict circulatory plasma OPG levels [49]. Therefore, it is likely that serum OPG concentration may constitute an important and specific CVD biomarker in T2DM. The use of serum RANKL as a CV biomarker is more controversial. It has been claimed for example that circulating RANKL levels exhibit no correlation with either advanced carotid atherosclerosis [49] or carotid intimal-medial thickness [44]. More recently however, both serum and tissue RANKL have been positively correlated with carotid calcification in atherosclerotic lesions [42]. With respect to T2DM, Gaudio *et al.* [44] have reported that circulating RANKL

Fig. 1

(a) Extensive vascular calcification of the femoral arteries on plain film radiograph, indicated by white arrows, adapted and reproduced with permission from Couri *et al.* [24] (<https://creativecommons.org/licenses/by/2.0/>), (b) computed tomography of the coronary arteries, with areas of calcification circled, adapted and reproduced with permission from Piers *et al.* [25] (<https://creativecommons.org/licenses/by/2.0/>).

levels were lower in diabetics than in control subjects, whereas O'Sullivan *et al.* [37] reported no change in plasma RANKL levels in T2DM. It has also been reported that RANKL expression is upregulated and localized to areas displaying medial arterial calcification in patients with charcot neuroarthropathy [21], whereas soluble RANKL has also been positively coassociated with well-known biomarkers of heart failure [50]. Interestingly, although it may not have intrinsic diagnostic value, Mohammadpour *et al.* [51] have proposed the OPG: RANKL serum concentration ratio as a biomarker for CAD. In their ischemic coronary disease study cohort, they noted a significant correlation between OPG/RANKL and CAC [51]. Overall however, based on recent clinical data, a definitive role for RANKL as a serum biomarker for T2DM/CVD remains inconclusive.

Therapeutic considerations

The dynamic nature of VC offers significant potential for clinical intervention in patients with type 2 diabetes manifesting CV complications. It is clear that the

dynamic osteoblastic pathways involving OPG, RANKL, and TRAIL represent ideal therapeutic targets for interference of the calcification process, but to date no treatment options are available for VC across the T2DM/CVD patient spectrum.

Recombinant osteoprotegerin therapy

Unsurprisingly, in view of its mechanism of action, OPG administration has been suggested as one potential treatment option for VC [52]. In mice, studies have shown that recombinant OPG fusion protein (Fc-OPG) can inhibit VC [18]. In this study, *ldlr*^{-/-} mice were fed an atherogenic diet alongside Fc-OPG administration; calcification was specifically inhibited with no effect on atherosclerotic lesion number or size.

Antireceptor activator of nuclear factor- κ B ligand therapy

Owing to the cross-over in molecular mechanisms between bone morphogenesis and VC, it is possible that a second prospective treatment for VC could be adapted

from currently existing osteoporosis therapy [52]. Osteoporosis is a systemic skeletal disease in which the level of bone resorption is greater than that of bone formation, leading to continuous bone degradation and ultimately resulting in low bone mass and fragility [53]. Denosumab, a human monoclonal antibody for RANKL, is used as a 6 monthly injection to treat osteoporosis and reduce fracture risk [52], although its effects on VC have not yet been fully assessed. Mimicking the natural actions of OPG, denosumab binds and neutralizes RANKL (but not TRAIL), attenuating its osteoclastic effects and allowing osteoblastic buildup of bone to ensue [54]. As RANKL promotes osteoblastic activity in VSMCs, anti-RANKL therapy could theoretically function to reduce the extent of calcification in the vasculature. In support of this theory, it has been demonstrated that denosumab reduced aortic calcium levels by half in a mouse model of osteoporosis [55], but contrastingly, the only corresponding human study completed to date has noted no influence of this therapy on aortic calcification progression over a 3 year-period [56]. It is possible that this disparity is owing to inconsistencies in calcification measurement, as Samelson *et al.* [56] utilized a semi-quantitative method (lateral spine radiographs) as opposed to the quantitative measurement of aortic calcium deposition employed by Helas *et al.* [55]. Furthermore, this study was a subanalysis of a larger trial initially completed to assess the effects of OPG administration on bone mineral density in osteoporotic postmenopausal women (2363 of 7808 patients). The therapeutic potential of anti-RANKL therapy for the treatment of VC therefore awaits further clinical investigation.

Tumor necrosis factor-related apoptosis-inducing ligand administration

Recombinant TRAIL administration to ApoE-deficient diabetic mice has been shown to significantly reduce atherosclerosis progression [29], whereas TRAIL delivery protects against diabetic vascular injury in rats [57]. Also of relevance, TRAIL deficiency appears to promote VC and diabetes *in vivo* [58]. Although these investigations appear promising, more robust human clinical studies are required to determine if TRAIL treatment directly contributes to an improved outcome in patients with CV complications.

Additional therapeutic possibilities

Bisphosphonates (pyrophosphate analogs) are a successful osteoporosis treatment and have been considered as a potential VC therapy option owing to their inhibitory effect on hydroxyapatite crystal formation [59]. Although animal studies have shown promise [60], human studies involving bisphosphonates and calcification have revealed mixed results [61,62]. Additionally, teriparatide, a shortened recombinant human parathyroid hormone also employed for osteoporosis treatment through its

effect on stimulating bone formation, has been shown to reduce VC in *ldlr*^{-/-} mice [63], but to our knowledge, there are no human data in relation to teriparatide and vascular disease. Statins, which have been routinely employed to lower blood cholesterol and prevent vascular complications associated with CVD and T2DM, have also been considered as a potential treatment option for VC, in view of their inherent pleiotropic properties [52]. In this respect, studies thus far have demonstrated conflicting results. Statin-treated patients were shown to reduce aortic stenosis [64], and to have a protective effect on VC in rats [65]. Additionally, statins have been shown to reduce levels of procalcific serum RANKL [66] and to increase anticalcific serum OPG. Elsewhere, it has been claimed that statins do not affect aortic stenosis with calcification [67], whereas a recent study has suggested that statins actually promote coronary atheroma calcification [68]. Further investigation is clearly warranted therefore to resolve this ongoing debate and determine if the pleiotropic effects of statins can successfully reduce VC. Glucagon like peptide-1 receptor agonists, a new injectable glucose-lowering agent working through the incretin system of the gastrointestinal tract, has shown in an *in-vitro* study using exenatide an attenuation of osteoblastic differentiation and calcification of SMCs in both a time-dependent and dose-dependent manner, alongside a decrease in the expression of RANKL [69]. Endothelin receptor agonists used to treat hypertension may also hold therapeutic value, as they have been shown to be effective against VSMC calcification *in vitro* [52] and also in rat models of VC [70]. In other recent studies, it has been observed that aortic calcification can be attenuated by a monoclonal antibody to interleukin-1 β (01BSUR, Novartis) in *ldlr*^{-/-} mice, highlighting a new potential therapy for VC that targets the link between CVD and inflammation [71].

Conclusion

VC is common, accelerated by diabetes, and is associated with poor CV outcomes, that is, increased morbidity and mortality. The OPG/RANKL/TRAIL system and the relationship between the three proteins appear to regulate and control the mechanisms underlying the pathogenesis of VC. Medications that can modify the OPG/RANKL/TRAIL system therefore offer an opportunity to slow down the progression of VC in patients with and without diabetes.

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Conflicts of interest

There are no conflicts of interest.

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