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For Debate

Vascular calcification and osteolysis in diabetic neuropathy—is RANK-L the missing link?

W. Jeffcoate[1]

[1] Foot Ulcer Trials Unit, Department of Diabetes and Endocrinology, City Hospital, Nottingham, UK

Abstract

Diabetic neuropathy is associated with osteopenia and calcification of vascular smooth muscle cells. These changes are most marked in patients with acute neuropathic osteoarthropathy (Charcot foot), in which osteopenia is universal and the prevalence of vascular calcification exceeds 90%. While it has been thought that both osteopenia and vascular calcification may be linked to sympathetic denervation with increased peripheral limb perfusion, the cellular mechanism was not clear. However, the recent recognition that the receptor activator of nuclear factor kappa B ligand (RANK-L)/osteoprotegerin (OPG) signalling pathway is central to the processes regulating bone turnover in a wide variety of medical conditions has raised the possibility that the same cytokines may be involved in the osteolysis which accompanies diabetic neuropathy. This is made more likely by the realisation that the RANK-L/OPG pathway is also thought to mediate the calcification of vascular smooth muscle cells in coronary and peripheral vascular disease. The circumstantial evidence underpinning this hypothesis is reviewed here, and it is suggested that the unregulated activation of RANK-L-mediated effects on bone and arteries may be triggered by the loss of nerve-derived peptides, e.g. calcitonin gene-related peptide, which normally exert a moderating influence on the pathway.

Keywords Amputation · Calcitonin gene-related peptide · Charcot · Diabetes · Foot ulcer · Neuropathy · OPG · Osteoporosis · RANK-L · Vascular disease

Introduction

The distal symmetrical neuropathy of diabetes is associated with arterial calcification and osteopenia. The link between these findings has hitherto been obscure, although both might be related to changes in blood flow caused by sympathetic denervation. Peripheral vascular resistance is reduced in peripheral neuropathy, leading to increased peripheral flow with a widened pulse pressure in patients without significant macrovascular disease. It was thought that these haemodynamic changes could initiate calcification in vascular smooth muscle cells (VSMCs), and the resultant loss of arterial compliance would cause further widening of the pulse pressure. The mechanism by which increased bone blood flow induced osteolysis was not known.

However, the recent discovery of the osteoprotegerin (OPG)/receptor activator nuclear factor kappa B ligand (RANK-L, or OPGL) signalling pathway has identified a cytokine system common to the processes both of vascular calcification and osteoporosis. Abnormal functioning of this system could explain why they occur together in diabetic neuropathy.

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W. Jeffcoate (✉)
Foot Ulcer Trials Unit,
Department of Diabetes and Endocrinology,
City Hospital, Nottingham, NG5 1PB, UK
E-mail: wjeffcoate@futu.co.uk
Tel.: +44-1158-405859, Fax: +44-1158-405859

Abbreviations: CGRP, calcitonin gene-related peptide · IAPP, islet amyloid polypeptide, amylin · OPG, osteoprotegerin · RANK-L, nuclear factor kappa B ligand · VSMC, vascular smooth muscle cell

Association of neuropathy with vascular calcification and osteopenia

Vascular calcification. The association between peripheral neuropathy and calcification of VSMCs is well recognised [1, 2, 3, 4, 5], although the causative nature of the link cannot be established in cross-sectional studies. Moreover, the demonstration of sympathetic denervation is dependent on preservation of vascular reactivity, and this is impaired when vessels are calcified. Nevertheless, amputees with diabetes have a greater prevalence of neuropathy and vascular calcification than non-diabetic control amputees [6, 7]. Vascular calcification is particularly marked in patients with acute neuropathic osteoarthropathy (acute Charcot), where it has been observed in 78 to 90% of cases [8, 9].

Osteopenia. The link between neuropathy and osteopenia is less clear, since it is confounded by the tendency for obesity to cause increased bone density in patients with Type 2 diabetes [10]. However, the increased prevalence of osteopenia has been established in Type 1 diabetes, and has been shown to correlate with neuropathy [11, 12, 13, 14]. This has been shown to be associated with increased flow of blood to bone [15]. The data in patients with Charcot foot are unequivocal: both neuropathy and osteopenia are universal [16, 17].

The OPG/RANK-L signalling pathway

Bone turnover. RANK-L is a transmembrane protein related to TNF, and interacts with the RANK receptor on pre-osteoclasts to transform them into mature osteoclasts, inducing bone resorption. RANK-L is expressed in a number of cell types, including monocytes/tissue macrophages, osteoblasts and activated T cells. Increased RANK-L expression has been defined in a wide variety of conditions characterised by osteolysis: osteoporosis (age-related and steroid-induced), malignant bone disease (especially myeloma), Paget's disease (juvenile and later onset), and rheumatoid arthritis [18, 19, 20, 21].

A member of the TNF receptor superfamily, OPG is a glycoprotein that is secreted by a number of cell types, including osteoblasts and activated T lymphocytes. It serves as a decoy receptor for RANK-L, effectively inactivating it when production is excessive. It is thought that the coordinated synthesis of RANK-L and OPG is an essential feature of bone remodelling, allowing the necessary balance between lysis and new bone formation. However, loss of the normal coordination between expression of RANK-L and OPG can lead to exaggerated bone loss in a wide variety of disease states.

Expression of OPG and RANK-L is modulated, directly or indirectly, by a large number of factors, including TNF-α, IL-1 and IL-17, other interleukins, sex steroids, growth hormone and IGF-1, macrophage colony stimulating factor (M-CSF, CSF-1), nitric oxide, leptin, fetuin-matrix Gla protein (MGP) complex, thyroid and parathyroid hormones, 1,25 dihydoxy vitamin D and lipoproteins [18, 19, 20, 21]. Most significantly, the signalling pathway is also affected by calcitonin and related hormones, calcitonin gene-related peptide (CGRP) and islet amyloid polypeptide (IAPP) [22, 23, 24].

Vascular calcification. The OPG/RANK-L signalling pathway also plays a key role in regulating the expression of bone matrix proteins in VSMCs, which is central to the process of vascular calcification in atherosclerosis. Thus if the factors that trigger RANK-L expression (and osteolysis) in bone are the same as those that trigger expression (and vascular calcification) in VSMCs, this could explain the long-recognised link between osteoporosis and increased cardiovascular risk [25]. Since OPG and RANK-L are expressed in T lymphocytes and presumably involved in immune modulation, they may also be integral to the accepted relationship between cardiovascular disease and serum markers of inflammation [25, 26].

RANK-L and OPG in human disease. Whereas RANK-L has a lytic effect in bone, in VSMCs it promotes calcification. The action of OPG is essentially protective (and thought to be predominantly reactive and compensatory) in both tissues. It follows that even though the actions of both substances work against each other, expression of both is elevated in the same conditions. Although the relationship between tissue expression and the concentrations circulating in blood is uncertain, it has been shown that serum concentrations of RANK-L and OPG are elevated in the diseases specified above as being associated with increased bone turnover.

The factors that might lead to macrovascular disease through over-expression of RANK-L presumably differ in different disease states. One of these factors may be increasing intravascular pressure. It has long been known that atherogenesis is accelerated by hypertension and exaggerated at sites exposed to the greatest forces during the systole, e.g. the bifurcation of the aorta and other major vessels. Once such calcification occurs, vessel compliance is restricted, leading to further widening of the pulse pressure and increasing systolic forces which exacerbate the tendency to medial calcification.

There have been few studies on the serum concentrations of OPG and RANK-L in conditions, other than osteoporosis, that are associated with increased cardiovascular risk. Nevertheless, it has been shown that serum OPG was higher in men with coronary artery disease than in men without [27]. It was noted in the same study that serum OPG was higher in peo-

ple with diabetes. Browner and colleagues found that serum OPG concentrations were higher in women with diabetes and that they correlated with cardiovascular risk [28]. Serum concentrations of OPG were also found to be higher in another small study of patients with Type 2 diabetes, and possibly associated specifically with microvascular complications [29]. There is evidence that the OPG/RANK-L system is involved in the accelerated vascular calcification of chronic renal failure [30], and it has been shown that it is modulated in vitro by inorganic phosphate [31]. A direct correlation was observed between serum OPG and the onset of aortic calcification in a five-year prospective study [32].

The possible role of the OPG/RANK-L signalling pathway in diabetic neuropathy

The involvement of this pathway in diabetic neuropathy is made probable by the otherwise unexplained association between neuropathy, vascular calcification and osteopenia in a disease in which circulating concentrations of OPG have been reported to be high. If confirmed, it is possible that the linking mechanism could be mediated through the changes in intravascular flow and pressure referred to above. There is little to substantiate the possibility that changes in flow alone could trigger osteolysis, although osteolysis is a well-recognised feature of other conditions associated with abnormal peripheral circulation, including paraplegia and reflex sympathetic dystrophy (complex regional pain syndrome-1). There is also recent evidence from experimental animals that the expression of RANK-L in bone cells is force-dependent [33]. Such a relationship would make teleological sense—in that osteolysis is a key step in bone healing, and it would clearly be advantageous, if it were induced by the increased blood flow that follows traumatic fracture. Traumatic fracture is associated with marked increases in expression of OPG and RANK-L [34].

OPG/RANK-L in Charcot foot. The Charcot foot represents an extreme model for the process linking diabetic neuropathy, osteolysis and vascular calcification. Neuropathy and osteolysis are universal in this condition, and evidence of vascular calcification on plain X-ray has been reported in up to 90%. Vascular dilatation in bone, as reflected in widening of the Haversian canals, has long been recognised to occur in syphilitic osteoarthropathy [35]. However, it is not known why acute Charcot foot should develop in a small subset of patients with diabetic neuropathy. It may reflect the severity of denervation and/or the extent of any associated macrovascular disease, both of which would limit the degree of resultant hyperaemia. But it is also possible that the process is triggered by other factors that exaggerate limb blood flow in a person already predisposed to it by neuropathy. Such factors might include minor trauma, previous foot ulcer and even surgical revascularisation [36]. Such a process would explain the asymmetry observed between affected and non-affected limbs; ipsilateral hyperaemia would be further augmented once any fracture or dislocation occurred in the Charcot foot.

It is suggested, therefore, that the vicious cycle that is integral to the pathogenesis of the Charcot foot is as much based on hyperaemia, as it is on increased force being applied to bone as a consequence of fracture and dislocation. Its dependence on increased blood flow explains why the process responds to rest (which will help to reduce it) and, possibly, to bisphosphonates [37, 38]. Bisphosphonates inhibit the action of osteoclasts, although not by impacting directly on the OPG/RANK-L system [39, 40], but their use also reduces local blood flow in other conditions associated with regional hyperaemia [41, 42]. If the long-term benefit of bisphosphonates is ever proven in the Charcot foot, it is as likely to be the result of this effect on local circulation as on their inhibition of bone resorption by osteoclasts.

Other interrelated processes

While changes in vascular flow may be the mechanism common to any increase in RANK-L expression that occurs in arteries and bone in diabetic neuropathy, this is likely to be compounded by other influences, especially as many factors are known to modulate the OPG/RANK-L pathway. Particular candidates are three related peptides: calcitonin, CGRP and IAPP. Calcitonin has long been recognised as a key factor in the process of bone remodelling and shares with OPG the capacity to inhibit bone resorption [43] by direct action on osteoclasts [23, 44]. It also inhibits osteoclast stimulation induced by RANK-L [22, 44]. CGRP, the second candidate, also inhibits osteoclast function [23] and stimulates bone formation [24]. As CGRP is also present in the nerve fibres which innervate bone [24], it is possible that neuropathy facilitates osteolysis by RANK-L through deficiency of CGRP and/or other nerve-derived substances. The fact that the third candidate, the related (and islet-derived) peptide IAPP, also inhibits osteclastic function may be linked to the observation that neuropathy-associated osteopenia is more clearly associated with Type 1 diabetes, in which secretion of IAPP is deficient.

Diabetic neuropathy and cardiovascular risk

Since neuropathy is associated with an increased incidence of vascular calcification, it may confer an independent increase in cardiovascular risk. While there is evidence that this is true [45], it is difficult to dissect

an effect of distal symmetrical neuropathy from a possible confounding effect on mortality from autonomic neuropathy and cardiac denervation [46], as well as from the effect of nephropathy. Given, however, that the vascular calcification associated with neuropathy would be characterised by widened pulse pressure, it is worth noting that widened pulse pressure has recently been shown to be an independent predictor of death in patients with renal failure [47, 48].

Implications

Full understanding of these processes is likely to have fundamental significance for diabetes care. At the very least it will allow the adoption of potential new markers for the risk and activity of the Charcot foot, as well the possibility that synthetic OPG might be therapeutically useful. In the shorter term, it might be more logical to consider treating patients with calcitonin rather than bisphosphonates. However, confirmation that the OPG/RANK-L signalling pathway is intimately involved, as suggested, in the pathogenesis of macrovascular disease in diabetes would encourage new speculation on the multiple interrelationships between microvascular and macrovascular complications, and on the interplay of immune mechanisms, nerve-derived peptides, lipoprotein metabolism and other metabolic processes.

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