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The pathogenesis of Charcot neuroarthropathy: current concepts

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The pathogenesis of Charcot neuroarthropathy (CN) has been poorly understood by clinicians and scientists alike. Current researchers have made progress toward understanding the cause of CN and possible treatment options. The authors review the current literature on the pathogenesis of this debilitating disorder and attempt to explain the roles of inflammation, bone metabolism, and advanced glycation end products.

Keywords: *Charcot neuroarthropathy*; *osteoclast*; *inflammation*; *RANKL*; *diabetic neuropathy*

Since its first description by Sir William Musgrave in 1703, the pathogenesis of Charcot neuroarthropathy (CN) has bewildered even the most astute physicians and scientists. In 1883, Jean-Martin Charcot described the '*pied tabétique*' or 'tabetic foot,' as tabes dorsalis was the most common cause of neuroarthropathy at the time (1). Diabetes mellitus has long surpassed syphilis as the leading cause of CN in the United States, and the prevalence of diagnosed CN in patients with diabetes is reported to be 0.08%–7.5% (2). Although rare, CN is one of the most destructive complications of diabetes, leading to subluxation, dislocation, deformity, and ulceration of the foot and ankle joints. A greater understanding of the pathogenesis of CN is needed to develop new treatment strategies. This review article aims to summarize the recent literature on the pathogenesis of CN at the molecular level and looks toward therapies that function to antagonize the pathologic mechanisms.

Uncontrolled inflammation and bone metabolism

Charcot postulated that neuropathic joints were caused by an abnormality of blood flow due to denervation: 'denervation will also be expressed by changes in the circulation or nutrition if it involves, in addition, nerve fibers which are vasomotor or trophic' (translation) (1). In addition, Charcot recognized the role of acute inflammation: 'the joints were inflamed, red and rather painful, similar to exacerbations of subacute rheumatoid arthritis' (translation) (3). Nearly 150 years later,

researchers have found molecular evidence to support Charcot's observation of acute inflammation. Indeed, it is the uncontrolled inflammation that results in the final common pathway for decreased bone density in CN: osteoclast–osteoblast imbalance. Under normal circumstances, bone undergoes a constant remodeling process, with an intricate homeostasis between bone resorption by osteoclasts and bone formation by osteoblasts. Gough and colleagues proved that excessive osteoclastic activity occurs in patients with acute CN (4). They studied four groups: patients with acute CN, chronic CN, diabetic controls, and non-diabetic controls. Using radioimmunoassay of blood samples from the dorsal venous arch, this group of researchers found a statistically significant increase in the pyridinoline cross-linked carboxy-terminal telopeptide domain of type 1 collagen, which is indicative of osteoclast activity, in the acute CN group ($p < 0.0001$). A similar increase in osteoblast precursors, carboxy-terminal propeptide of type 1 collagen was not found, indicating a higher concentration of osteoclasts than osteoblasts. In addition, the patients with acute CN showed a significant increase in levels of alkaline phosphatase, a marker of bone turnover.

The role of inflammation in upsetting osteoclast–osteoblast homeostasis was investigated by Baumhauer et al. (5). The investigators stained 20 surgical bone specimens of Charcot patients with hematoxylin and eosin (H&E), interleukin-1 (IL-1) antibody, tumor necrosis factor (TNF) alpha antibody, and interleukin-6 (IL-6) antibody. These inflammatory cytokines lead to

bone resorption by promoting osteoclast recruitment, proliferation, and differentiation. Osteoclasts demonstrated a moderate pattern of staining for TNF-alpha and IL-1, and a diffuse pattern of staining for IL-6. These results suggest that osteoclasts express inflammatory cytokines during the acute and reparative phases of CN. H&E staining also showed the presence of excessive numbers of multinucleated osteoclasts in lacunae surrounded by lamellar bone. Uccioli and colleagues further elucidated the role of inflammation in the Charcot process as they characterized the cytokine phenotype of monocytes in patients with acute CN (6). They studied three groups: patients with acute CN, diabetic patients with neuropathy, and normal control subjects. In patients with acute CN, they found monocytes expressing increased amounts of the proinflammatory cytokines TNF-alpha, IL-1 beta, and IL-6 as well as decreased amounts of the anti-inflammatory cytokines Il-4 and Il-10. Furthermore, monocytes from acute CN subjects had a marked increase in the percentage of positive cells and intensity of expression of the costimulatory surface molecules CD40, CD80, and CD86. In contrast, there was no statistically significant difference in these surface molecules among the diabetic control and healthy control groups ($p > 0.05$). The inability to end the cycle of aggressive inflammation that is so characteristic of CN was explained by a resistance to monocyte apoptosis conferred by IL-1 beta and TNF-alpha. The results of this study stimulate one to consider anti-TNF-alpha therapy as a potential treatment for CN.

The role of receptor activator of nuclear factor-kappa B ligand (RANKL)

The presence of proinflammatory cytokines alone does not account for the entire influx of osteoclasts in CN. Receptor activator of nuclear factor-kappa B ligand (RANKL) has been studied extensively for its role in activating osteoclasts in diabetic CN. RANKL is an important mediator of osteoclastogenesis and is essential in osteoclast formation and modulation (Fig. 1). The antagonist of the RANKL pathway is osteoprotegrin (OPG). Jeffcoate suggested that disruption of the RANKL/OPG pathway is responsible for both vascular smooth muscle calcification and the osteopenia seen in CN (7). Three years later, Mabilleau and colleagues performed an elegant study that demonstrated the role of RANKL in CN and also suggested that there may be a RANKL-independent pathway (8). Osteoclast formation was assessed in peripheral blood samples from nine diabetic Charcot patients, eight age-matched diabetic controls, and eight age-matched non-diabetic control patients. Three isolates were obtained: one in the presence of macrophage-colony stimulating factor (M-CSF) alone, one in the presence of M-CSF and RANKL, and one containing M-CSF, RANKL, and OPG. They found

a statistically significant increase in the formation of osteoclasts in diabetic Charcot patients when compared with diabetic control and non-diabetic control patients ($p = 0.008$) in cultures with M-CSF alone. Percentage of area bone resorption in Charcot patients was four times higher than in healthy controls ($p = 0.005$) and 2.9 times higher than in diabetic controls ($p = 0.008$). The addition of RANKL to the cultures yielded a significant increase in osteoclastic resorption in the Charcot group when compared with the diabetic and healthy control groups ($p < 0.0001$). Finally, the addition of OPG caused a greater decrease in resorption in the diabetic and healthy control groups than in the Charcot group, implicating that there is a RANKL-independent pathway of bony destruction in CN. This study demonstrated unequivocally that osteoclast precursor cells in acute Charcot patients are 'primed' to become osteoclasts with aggressive behavior. The authors of this study suggest that the increased levels of circulating proinflammatory cytokines, TNF-alpha, IL-6, and IL-8 induce osteoclast formation independent of RANKL. Indeed, inflammation is the final common pathway in the pathogenesis of CN.

To attempt to address the osteoclast–osteoblast imbalance, there has been research to evaluate the use of bisphosphonates in CN. Pitocco et al. performed a Level 1 study of 20 patients in which the treatment group received 70 mg of alendronate once weekly, and the control group received placebo (9). Both groups were prescribed standard off-loading methods. While these researchers found significant reductions in hydroxyproline and serum C-terminal telopeptide of type 1 collagen (ICTP), markers of bone turnover, they did not report differences in the resolution of clinical symptoms. They did, however, demonstrate a reduction in the serum levels of insulin-like growth factor 1 (IGF-1) in the treatment group. IGF-1 causes vasodilatation, adding to the hyperemia that already exists in CN. This finding prompted the same group of researchers to study the relationship between IGF-1, neuropathy, inflammation, and the RANKL system (10). Bisphosphonates may decrease IGF-1 and help regulate RANKL, but their clinical efficacy remains to be proven.

The influence of calcitonin gene-related peptide (CGRP) and nitric oxide

How is the expression of RANKL increased in patients with CN? Aside from the supposition that inflammation itself induces RANKL, there is research that supports a lack of negative-feedback mechanisms in patients with diabetes. Peripheral and autonomic neuropathy can minimize the release of the neuropeptide calcitonin gene-related peptide (CGRP), which antagonizes the expression of RANKL. CGRP inhibits osteoclast motility, recruitment, and differentiation (11). Associated with
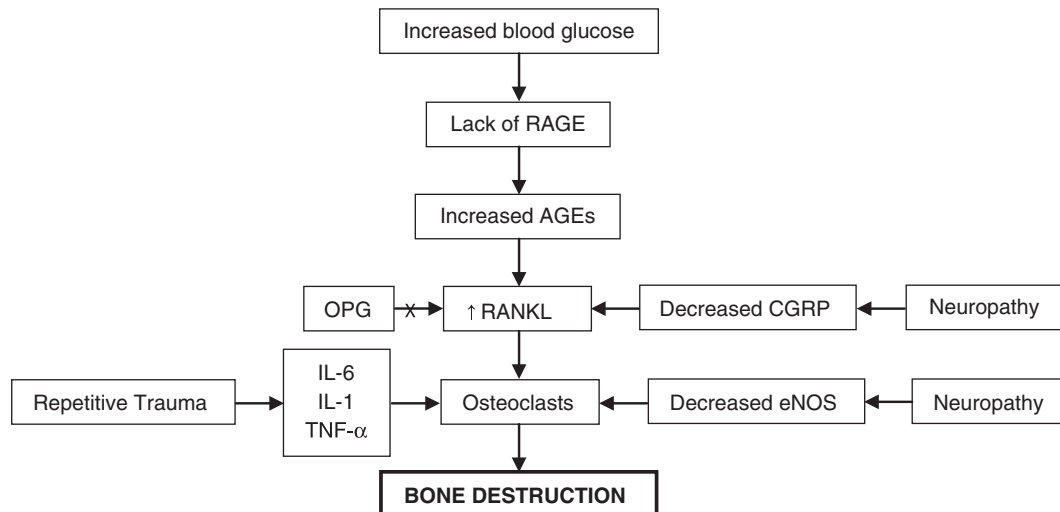
Fig. 1. The role of RANKL in Charcot neuroarthropathy.

blood vessels, CGRP is produced in the hypothalamus and found in the periosteum and bone marrow. Experiments utilizing fractures in rat femora demonstrated that CGRP increases at the site of the fracture gap, suggesting that neuropeptides play an active role in bone remodeling (12). With a lack of CGRP, osteoclasts are recruited by RANKL in an unchecked fashion. La Fontaine and colleagues substantiated the CGRP hypothesis with their study in 2008 (13). They performed immunohistological studies in bone samples of three groups: diabetic patients without neuropathy, diabetic patients with neuropathy, and diabetic patients with stage 2 or 3 CN. Samples of bone were collected during reconstructive operations, and patients with history of ulceration, osteomyelitis, or end-stage renal disease were excluded. They found a trend toward significance in comparing CGRP expression, with the Charcot group having the least amount of CGRP. Additionally, these researchers looked at the relative amounts of endothelial nitric oxide synthase (eNOS), an isoenzyme that regulates nitric oxide production. Nitric oxide is a free radical that suppresses osteoclasts. Immunohistochemical studies found a statistically significant decrease in eNOS in the Charcot bone compared with the other two groups ($p = 0.008$); thus, lack of eNOS is another breakdown in the negative-feedback mechanisms that are normally in place for osteoclast modulation and proliferation.

Advanced glycation end product (AGE) accumulation

Another mechanism by which RANKL, and thus osteoclast function, is increased is by accumulation of advanced glycation end products (AGEs). The formation of AGEs is driven by hyperglycemia and primarily affects collagen in tissues with the slowest turnover, such as cortical bone (14). AGEs have been found to increase

RANKL activation as well as induce osteoblast apoptosis (15). In patients with diabetes, there is increased formation of AGEs but a lack of a receptor for AGEs (RAGE). Witzke and colleagues designed a cross-sectional study in which they enrolled 80 male subjects: 30 healthy controls, 30 diabetic patients without Charcot, and 20 diabetic patients with stage-2 CN (14). They found a significant reduction in calcaneal stiffness in the patients with CN ($p < 0.01$). In all subjects, there was a positive correlation between calcaneal bone stiffness and RAGE concentration, indicating that RAGE is protective against osteoclastic resorption. CN patients had RAGE values that were 86% lower than control subjects and 50% lower than diabetics without CN ($p < 0.05$). In addition, these researchers found an elevated level of osteocalcin, a marker of bone turnover, in CN patients. In summary, there was a linear relationship between impaired AGE defense (lack of RAGE), increased bone turnover, and reduced bone stiffness. This study suggests compelling evidence that drugs that increase RAGE levels, such as ACE-inhibitors, statins, and glitazones, may be useful in preventing or suppressing CN.

Conclusion

With an increasing prevalence of diabetes, there will be a rising incidence of CN, along with a higher rate of the associated devastating foot and ankle complications. The exact pathogenesis of CN remains elusive; however, it is known that sensory and autonomic neuropathy are prerequisite to begin the process of uncontrolled inflammation through proinflammatory cytokines TNF-alpha and interleukins, unchecked activation of RANKL, and the resulting imbalance of osteoclasts and osteoblasts (16). Neuropathy decreases the available amounts of CGRP and eNOS that in turn activate RANKL. Elevated glucose levels form AGEs, and the lack of

RAGE leads to an increase in RANKL. These mechanisms that have been implicated in the pathogenesis of CN are the targets of therapeutic agents, including bisphosphonates, anti-TNF therapy, ACE-inhibitors, statins, and glitazones.

Conflict of interest and funding

References

1. Charcot J-M, Féré C. Affections osseuses et articulaires du pied chez les tabétiques (pied tabétique). Archives de Neurologie 1883; 6: 305–19.
2. Bowker JH, Pfeifer MA. Levin and O'Neal's the diabetic foot. 7th ed. Philadelphia: Mosby; 2008.
3. Charcot J-M. Sur quelques arthropathies qui paraissent dépendre d'une lésion du cerveau ou de la moëlle épinière. Arch Physiol Norm Pathol 1868; 1: 161–78.
4. Gough A, Abraha H, Li F, Purewal TS, Foster AVM, Watkins PJ, et al. Measurement of markers of osteoclast and osteoblast activity in patients with acute and chronic diabetic Charcot neuroarthropathy. Diabetic Med 1997; 14: 527–31.
5. Baumhauer JF, O'Keefe RJ, Schon LC, Pinzur MS. Cytokine-induced osteoclastic bone resorption in Charcot arthropathy: an immunohistochemical study. Foot Ankle Int 2006; 27: 797–800.
6. Uccioli L, Sinistro A, Almerighi C, Ciaprini C, Cavazza A, Giurato L, et al. Pro-inflammatory modulation of the surface and cytokine phenotype of monocytes in patients with acute Charcot foot. Diabetes Care 2010; 33: 350–5.
7. Jeffcoate W. Vascular calcification and osteolysis in diabetic neuropathy—is RANK-L the missing link? Diabetologia 2004; 47: 1488–92.
8. Mabilleau G, Petrova NL, Edmonds ME. Increased osteoclastic activity in acute Charcot's osteoarthropathy: the role of receptor activator of nuclear factor-kappaB ligand. Diabetologia 2008; 51: 1035–40.
9. Pitocco D, Ruotolo V, Caputo S, Mancini L, Collina C, Manto A. Six-month treatment with alendronate in acute Charcot neuroarthropathy. Diabetes Care 2005; 28: 1214–5.
10. Pitocco D, Collina MC, Musella T, Ruotolo V, Caputo S, Manto P, et al. Interaction between IGF-1, inflammation, and neuropathy in the pathogenesis of acute Charcot neuroarthropathy: lessons from alendronate therapy and future perspectives of medical therapy. Horm Metab Res 2008; 40: 163–4.
11. Akopian A, Demulder A, Ouriaghli F, Corazza F, Fondu P, Bergmann P. Effects of CGRP on human osteoclast-like cell formation: a possible connection with the bone loss in neurological disorders? Peptides 2000; 21: 559–64.
12. Irie K, Hara-Irie F, Ozawa H, Yajima T. Calcitonin gene-related peptide (CGRP)-containing nerve fibers in bone tissue and their involvement in bone remodeling. Microsc Res Tech 2002; 58: 85–90.
13. La Fontaine J, Harkless LB, Sylvia VL, Carnes D, Heim-Hall J, Jude E. Levels of endothelial nitric oxide synthase and calcitonin gene-related peptide in the Charcot foot: a pilot study. J Foot Ankle Surg 2008; 47: 424–9.
14. Witzke KA, Vinik AI, Grant LM, Grant WP, Parson HK, Pittenger GL, et al. Loss of RAGE defense: a cause of s neuroarthropathy? Diabetes Care 2011; 34: 1617–21.
15. Alikhani M, Alikhani Z, Boyd C, MacLellan CM, Raptis M, Liu R, et al. Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. Bone 2007; 40: 345–53.
16. Jude EB, Boulton AJ. Update on Charcot neuroarthropathy. Curr Diab Rep 2001; 1: 228–32.

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