Neurotrophins and Peripheral Neuropathies

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Peripheral neuropathy is a common disorder seen in general neurology and neuromuscular speciality clinics. Treatment options directed at the underlying cause can only be offered in a handful of conditions, such as those with possible autoimmune etiology. The remainder fall into the idiopathic or genetic category with no known treatment. This review surveys the evidence supporting the rationale for the therapeutic use of neurotrophins and other neurotrophic factors in these disorders in relationship to the underlying pathobiological process. Previous clinical trials are assessed, and increasingly better understood and appreciated therapeutic potential of neurotrophins is emphasized.

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INTRODUCTION

Neurotrophins are involved in the support, survival, growth and maintenance of specific neuronal populations. Because of the specificity of growth factors for particular sets of neurons, it is almost intuitive that targeted growth factor delivery to key degenerative sites should be considered the best therapeutic option. This straightforward approach will restrict the therapeutic use of nerve growth factor (NGF) for only small-fiber sensory neuropathy, while the brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and neurotrophin 3 (NT-3) should be used for neuropathies involving large fibers (A-β and A-α nerve fibers) that are responsible for proprioception, vibratory sensation, muscle-stretch reflexes and muscle strength. A combination treatment should be advocated for conditions affecting all fiber sizes. However, an accumulating body of evidence now indicates that neurotrophins serve a variety of other functions, including effects on glia and other non-neuronal tissues. Neurotrophins are more versatile than previously realized, as evidenced by their direct effects in synaptic transmission, plasticity and their convergence role through interactions with other receptors and ion channels (9). With continuing research in this area, many of the remarkable actions of neurotrophins may be exploited for therapeutic use in peripheral nerve disorders, including the management of neuropathic pain. This review will include some of the preclinical studies and earlier clinical trials with neurotrophins and also highlight other neurotrophic factors relevant to peripheral neuropathies.

Although early clinical trials with neurotrophins have led to disappointing results, it is a realistic expectation that the potential therapeutic use of neurotrophins in peripheral neuropathy will be revisited. Further research should provide better information on the specific biological effects and the underlying mechanisms involved in their specific roles regarding axon regeneration, myelination and axon–Schwann cell (SC) interactions, which should set a solid stage for designing future clinical trials (12, 22, 63).

RATIONALE FOR NEUROTROPHINS IN THERAPY OF PERIPHERAL NEUROPATHIES

During the last decades, significant advances have been made in understanding the pathobiology of peripheral nerve disorders. The major challenge that investigators and clinicians are facing now is to devise effective treatments for these disorders. Peripheral neuropathies are subdivided into three groups according to the primary anatomical site of involvement: (i) cell body disease (neuronopathy); (ii) myelin disease (myelinopathy); and (iii) distal nonterminal axonopathy or dying back neuropathy, which typically affects the distal parts of the longest axons (length-dependent distal axonal disease or distal axonal neuropathy) and the clinical disability directly correlates with the degree of distal axonal loss. The focus in this review will be on the potential therapeutic use of neurotrophins in the third group, which represents the most common neuropathies. The causes are heterogeneous, affect both axons and SCs and can be either acquired or inherited.

The long-term outcome of a length-dependent distal axonal disease depends on two seemingly opposing, but intimately associated, pathobiological processes: (i) the degree or rate of axonal degeneration, progressing centripetally toward the cell body; and (ii) the ability of the nascent axon tips to regenerate efficiently. In conditions such as diabetic or hereditary neuropathy, where there is defect in regeneration, a slow progressive course is seen, because the ongoing degeneration, even at a low rate, will remain unopposed. One strategy to alter these processes would be to improve the efficiency of regeneration, which could potentially bring neurotrophins from bench to bedside.

Neurotrophins, SCs and axonal growth. Recent studies revealed that the regeneration outcome of peripheral nerve is poor after prolonged axotomy and denervation (37, 81, 82), a model that simulates a chronic distal axonal neuropathic process

in humans. SCs have the ability to survive several months after nerve transection, although their number gradually declines (23, 24, 38, 86). The ability of denervated SCs to survive is crucial for nerve regeneration, as SCs provide both growth factors and basal lamina scaffoldings that promote axonal growth. From embryo day 18 onward, SCs manage to acquire the ability to survive without axonal contact by establishing an autocrine survival loop, which is absent in the SC precursors that solely depend on axonal neuregulin-1 for survival (35, 48, 83, 84, 89). IGF-2, NT-3 and platelet-derived growth factor-BB (PDGF-BB) have been identified as major components of the autocrine loop (35, 48, 83, 84, 89). Prolonged denervation, however, could lead to decreased regeneration capacity because of reduction in the expression of regeneration-associated SC molecules (neurotrophic factors and their receptors) and the eventual atrophy of the denervated SCs and the breakdown of the bands of Bungner, with their SC basal lamina scaffoldings. Therefore, for efficient regeneration, SCs distal to the axonal injury site must remain in a growth-supportive mode for prolonged periods or they have to be transformed into a competent premyelinating state to initiate and complete myelination. Transforming denervated SCs into a competent state in chronic neuropathies poses great challenges, yet studies exploring the efficacy of autocrine survival loop growth factors, alone and in combination, could be a starting point.

Another rationale for the potential therapeutic use of neurotrophins is their proposed role in stabilizing the axonal cytoskeleton locally by inducing neurofilament phosphorylation when axon sprouts become enwrapped by SCs at the early stages of regeneration-associated myelination. SCs are known to exert a significant local influence on axon caliber by modulating neurofilament phosphorylation (14–16, 96). The phosphorylated state of neurofilaments appears to result from highly localized SC–axon interactions and is greatly influenced by the phenotype of SCs. Neurofilament hypophosphorylation leads to increased neurofilament packing density, the common pathology in different subtypes of Charcot–Marie–Tooth (CMT) diseases and CMT mice models (1–3, 7, 14, 16, 43, 67–70, 74, 96). Studies of xenografts from patients with peripheral myelin protein 22 (PMP22) duplications, deletions and missense mutations as well as those with connexin 32 (Cx32) missense mutations have shown that alterations in axonal caliber and neurofilament density develop at an early ensheathment stage of myelination, before myelin compaction (68–70). Moreover, in the central nervous system, ensheathment of axons by oligodendrocyte processes that do not form myelin because of mutations in myelin protein genes still increase axon caliber and neurofilament density (74). These observations strongly indicate that SC ensheathment alone provides the signal that induces axonal neurofilament organization and caliber changes, and emphasize the need to focus on the ensheathment stage of early myelination in exploring the role of neurotrophins in regeneration. Furthermore, primary SC genetic defects may interfere with regeneration. Studies of xenografts with SCs harboring PMP22 deletion, duplication or a specific PMP22 point mutation (V30M) revealed an intimate association between the delay of the onset of myelination (a temporary halt of the premyelination state) and impairment in the growth capacity of nude mice axons (an impairment of axon tip elongation) engulfed by mutant SCs (69–71). Studies of xenografts examined at 2 weeks showed that SCs that are due to myelinate envelop the abnormally enlarged axons but do not go to the next step of compacted myelin formation as if their ability to respond to organizational and metabolic challenges of myelination is impaired. Axon tips become "frustrated growth cones" and eventually undergo vacuolar degeneration.

Studies from this laboratory tested the ability of mutant SCs to respond to exogenous NT-3 in two different paradigms: a xenograft model of SCs with the common PMP22 duplication of CMT1A and naturally occurring animal model, *TremblerJ* (Tr^J) mice, which carry a point mutation in the PMP22 gene. NT-3 abolished frustrated growth cones, significantly improved axonal regeneration and the associated myelination process in both models (72). Moreover, ultrastructural morphometric analysis of neurofilament density distribution in grafted segments showed a significant shift toward normal range compared with the untreated group. In contrast to the dramatic improvement with NT-3 treatment, BDNF, which is not part of the SC survival loop, showed no effect upon axonal growth or neurofilament cytoskeletal pathology. In addition, in the Tr^J model, which also displays impaired regeneration, NT-3 resulted in a significant increase in SC number in the crushed, as well as intact, sciatic nerves, suggesting that NT-3 improved competency and availability of SCs for efficient nerve regeneration (72).

These results strongly indicate that exogenous NT-3 can compensate for the SC defects in CMT1A by reversing the neurofilament density toward normal range, suggesting enhanced neurofilament phosphorylation in the axon. This might be realized by retrograde transport of NT-3 to the cell body through the NT-3 receptor TrkC. Alternatively, NT-3 might act locally by activating signaling pathway(s) at the paranodal region as well as at the growing axon tips. The latter hypothesis appears more plausible by taking into account the fact that neurofilament phosphorylation largely occurs post-translationally in axons, which implies that signals from SC, either through direct contact or paracrine factors such as NT-3, must be transduced and amplified through axonal signal transduction pathways. Evidence for this possibility comes from *in vitro* studies showing the ability of NT-3 to phosphorylate neurofilament protein-H (NF-H) (88). Phosphorylation of NF-H and NF-L is necessary for forming well-developed crossbridges, straight and at constant intervals (21), and important for the growth and stabilization of the elongating axons (10, 55, 98). It should be noted that whereas NT-3 improves survival and competency of "sick" mutant SCs, it does not alter functional recovery following crush injury in normal animals, resulting in only slightly more axons than in untreated controls or NGF-treated animals (97).

Neurotrophins and neuronal cell body. Another rationale for the therapeutic use of neurotrophins in length-dependent distal axonal neuropathy is to interfere with the cell body response to the chronic axotomy state. Decreased axon caliber is a prominent and frequent abnormality in peripheral nerve disease with late conse-

quences of secondary segmental demyelination. Length-dependent distal axonal disease state, in which the axon tips are no longer in touch with their target, has similarities to experimental axotomy models that result in a stereotypic pattern of morphological responses as well as alterations in protein synthesis in the cell body (30, 31, 59). Somatofugal axonal atrophy arising from decreased neurofilament content in the axon is a well-recognized consequence of axotomy and can be reversed with neurotrophins. Studies have shown that axotomy-induced axonal atrophy and decreased neurofilament content in the proximal axon can be reversed by exogenous NGF and, conversely, NGF antiserum administered into the foot pad of normal animals produces somatofugal axonal atrophy in NGF-responsive sensory fibers in lumbar dorsal root ganglia (DRG) neurons, suggesting that NGF regulates axonal caliber in mature rats (20). Interruption of the retrograde transport of target-derived supply of NGF appears to initiate some components of the cell body reaction to axotomy, and it is suggested that delivery of NGF to the proximal stump reduces axonal atrophy by preventing the down-regulation of neurofilament synthesis in NGF-responsive sensory neurons (20). This is supported by studies demonstrating reversal of the axotomy-induced reduction of neurofilament mRNA in DRG neurons that express high-affinity NGF receptors following intrathecal infusion of NGF (92). In contrast, NGF application to the proximal stump inhibits the up-regulation of growth associated protein (GAP)-43 (52) while glial cell line-derived neurotrophic factor (GDNF) application results in a significant increase in motor neuron size and GAP-43 mRNA expression (47). GDNF, the first member of the GDNF family ligands (GFLs), was initially identified as a highly specific neurotrophic factor for midbrain dopaminergic neurons (6).

Studies have provided direct and quantitative evidence that long-term continuous treatment with exogenous GDNF significantly increased the number of motoneurons, which regenerate their axons, completely reversing the negative effects of chronic axotomy. A combination of exogenous GDNF and BDNF on motor axonal regeneration was significantly greater than either factor alone, and this effect was most pronounced following long-term continuous treatment (8). GDNF, a potent neurotrophic factor for motor neurons, is up-regulated in SCs following axotomy and is retrogradely transported to the motor neuron cell bodies (25, 32, 56). A decline in the inability of SCs to maintain GDNF support for both motor and sensory neurons correlates well with the failure of axonal regeneration into chronically denervated nerves (32). In a recent study, regeneration through a chronically denervated nerve was accomplished by transplanting neural stem cells overexpressing GDNF into the chronically denervated distal nerve (27). It is important to note that GFLs and neurotrophins activate common intracellular signaling pathways through their receptor tyrosine kinases and this feature could be beneficial for developing effective treatment protocols. In augmenting nerve regeneration, it is likely that both GDNF and neurotrophins could feed into the same signaling pathways as shown by the ability of NGF to activate Ret (a member of the receptor tyrosine kinase (RTK) superfamily), the signaling component of the multisubunit GDNF receptor in sympathetic neurons (90). This inter-RTK signaling is shown to take place both *in vitro* and *in vivo* by a mechanism, which is independent of GFLs and GDNF-family-receptor-α (GFRα) coreceptors. Therefore, NGF-dependent Ret phosphorylation could result in augmented growth, metabolism and gene expression in postnatal neurons (90).

In experimental animal models of diabetes and drug-induced neuropathies, the electrophysiological consequence of axonal atrophy, slowed nerve conduction, is shown to improve with neurotrophins in a specific target neuron-dependent manner. For example, in galactose-fed rats with axonal atrophy and nerve conduction deficit, exogenous BDNF treatment attenuated motor nerve conduction deficits in the sciatic nerve without a similar effect on the sensory fibers (50). Direct supply of GDNF to the axotomized nerve or intrathecal application resulted in a significant improvement in the motor nerve conduction velocity in a dose- and time-dependent manner (53). In streptozotocin-diabetic rats, NT-3 is reported to show attenuation of the axonal atrophy and associated tendency for increased NF-H phosphorylation in large fibers of the distal sensory nerves (sural) and corrected sciatic nerve conduction deficit, although it was without effect on spinal roots upon atrophy and NF-H subunit phosphorylation (51). NT-3 also restored the reduced H-reflex-related sensory nerve conduction velocity deficit in cisplatin as well as pyridoxine-induced peripheral neuropathy in rats (18, 28). In addition, abnormality of the cytoskeletal distribution of neurofilaments induced by cisplatin toxicity is corrected with NT-3 treatment (18). NT-3 at high doses, pumped directly onto the cut nerve stump, largely prevented the axotomy-induced marked slowing of conduction velocity in the sensory fibers; lower doses were less effective, and neurotrophin-4/5 (NT-4/5) had no effect. Conversely, sequestration of NT-3, the high-affinity ligand for TrkC, resulted in axotomy-like physiological effects, slowing both motor and sensory nerve conduction velocity, while sequestration of trkB ligands (BDNF and NT-4/5) showed significant slowing of motor but not sensory nerve conduction (54). A member of the neuregulin family of growth factors, recombinant human glial growth factor 2 (rhGGF2) has also shown a protective effect against cisplatin-induced deterioration of the sensory nerve conduction velocities in the rat, in a dose-dependent manner (85). GGF was first identified as a potent SC mitogen (36).

Effects of neurotrophins on neurofilament phosphorylation are not yet fully explored. However, it was shown that in primary cultures of embryonic rat cortical neurons, BDNF and NT-3 are able to stimulate phosphorylation of NF-H (88). In PC12 cells, NGF is shown to induce NF-M and NF-H expression and phosphorylation; extracellular signal-regulated kinases 1 and 2 (ERK 1/2)/mitogen-activated protein kinase (MAPK) and jun N-terminal kinase (JNK) pathways are responsible for the expression as well as phosphorylation of NF-H (11, 39, 93). The carboxyl terminal tail domain of NF-M is shown to be essential for the radial growth and cytoskeletal architecture of axons and it is suggested that a possible function of highly phosphorylated tail domains of NF-M and MF-H is to establish crossbridges between neurofilaments, which are involved in both organization and stabilization of neurofila-

ment cytoskeleton (61, 98). Collectively, these studies strongly suggest that neurotrophins are required for the maintenance of normal functional properties of peripheral neurons and deserve serious consideration in developing treatment protocols for peripheral neuropathies. Signal transduction pathways involved in the biological actions of the neurotrophins are discussed in detail in the accompanying article by Twiss et al (91).

Neurotrophins and external microenvironment of nerve. Vascular endothelial growth factor (VEGF), which belongs to the family of endothelial cell-specific angiogenic factors, also has relevance to the pathophysiology and treatment of neuropathies. Angiogenesis plays an essential role in nerve regeneration by providing access to endoneurium for hematogenous macrophages to clear up the debris in damaged nerves and to carry oxygen and nutrients, which are essential for the elongation of neurites and proliferation of SCs. A role for VEGF in regeneration was suggested, with studies illustrating that VEGF stimulates SC invasion and neovascularization of acellular grafts, used to bridge a gap in the sciatic nerve (78, 79). Interdependence between increased vascularization and enhanced regeneration was shown within an acellular conduit in axotomized rats (29).

VEGF was also shown to improve the capacity of the peripheral nerves to regenerate in experimentally induced diabetes mellitus (75). Hyperglycemia causes various metabolic defects that provoke vasoconstriction and reduce endoneurial blood flow, resulting in nerve hypoxia. In the rat model of type I diabetes, VEGF expression is increased in the cell bodies and axons of the DRG and in the sciatic nerve, presumably as a result of ischemia (73). In a streptozotocin-induced diabetic neuropathy model, the nerve blood flow was markedly reduced, correlating with a reduction in the number of vessels. A severe peripheral neuropathy developed in parallel, which was reversed by intramuscular gene transfer of a plasmid carrying VEGF1 or VEGF2. In contrast, vascularity and blood flow in the nerves was unchanged, implicating VEGF in neuroprotection, in addition to its well-recognized angiogenic activities (64, 75, 80).

Recent studies provide new evidence of possible interdependent relationships between aging, VEGF, angiogenesis and nerve regeneration (60). These findings suggest that vascular abnormalities might play a role in the idiopathic neuropathy of aging with potential clinical implications. In cases with an axonal type of neuropathy, severe neuropathic changes were associated with a decreased epineurial blood vessel number and a simultaneous, relative increase in the endoneurial blood vessel density (44). In the chronic ischemic neuropathy of elderly with nondiabetic atherosclerotic peripheral vascular disease, microvascular alterations in sural nerves were described, suggesting that they could play a key role in its development (46). These observations position VEGF as a favorable candidate for clinical trials. In fact, a preliminary clinical study carried out in patients with chronic ischemic neuropathy reports improvement with intramuscular VEGF gene transfer (77). A clinical trial evaluating the safety and efficacy of VEGF gene transfer in diabetic patients with sensory neuropathy is currently being carried out (34).

Neurotrophins and neuropathic pain. Painful sensory neuropathy has many causes. In one subtype referred to as "idiopathic small-fiber painful sensory neuropathy," the small myelinated and nociceptive C (unmyelinated) nerve fibers are mainly affected. Studies indicate that this condition represents the most common type of painful sensory neuropathy in patients older than 50 years of age. In another group of neuropathies associated with pain, the discomfort is caused in part by damage to small nerve fibers, but large nerve fibers that are responsible for proprioception, vibratory sensation, muscle-stretch reflexes and muscle strength are also affected. The distinction between the two subtypes of painful sensory neuropathies is important, not only in searching for an underlying cause but also for the development of appropriate potential neurotrophic treatments in future trials. Irrespective of the subtype of neuropathy, the pain generated by damage to small nerve fibers is debilitating and responds poorly to current treatment regimen (49).

Studies using several animal models of chronic pain have been instrumental in exploring the complex pathophysiological mechanisms underlying neuronal excitability and the resulting neuropathic pain syndromes and the involvement of neurotrophins, NGF, BDNF, NT-3 and the GDNF family in this process [reviewed in detail in (66)]. However, it should be kept in mind that these models have shortcomings in considering the complexity of pain syndromes verbalized by the patients. Pain can occur without provocation (ie, stimulus-independent, as with burning and paresthesias accompanying small-fiber neuropathies) or can be stimulus-evoked (eg, hyperalgesia in response to noxious stimuli or allodynia induced by non-noxious stimuli). The recent discovery that BDNF up-regulation in the DRG and spinal cord contributes to tactile allodynia suggested new treatment strategies (58). Adenosine triphosphate-stimulated spinal microglia induces neuropathic pain by signaling to lamina I neurons. BDNF is a crucial signaling molecule between microglia and neurons in this process (13). Blocking BDNF receptor signaling in a rat model of nerve injury prevented allodynia, demonstrating that BDNF is required for neuropathic pain. It is proposed that this finding holds promise for the development of therapies that block BDNF signaling. However, it was argued that these changes in the spinal cord are secondary to the pathophysiological changes in the first-order sensory neurons. Dramatically increased BDNF expression in the DRG neurons could be regulated by distinct activation of MAPK in pain states evoked by different mediators and pathological conditions, and, therefore, blocking BDNF in sensory neurons could represent a new approach to treating neuropathic pain (57). Another promising recent development in pain treatment is the emergence of a novel class of pain drugs based on antagonism of NGF (26). Studies have shown that selective antagonism of NGF is highly effective in animal models of acute and chronic pain states. A variety of novel approaches are currently being developed to antagonize NGF, including NGF "capture," blocking the binding of NGF to TrkA and inhibiting NGF signaling. It is claimed that NGF antagonism is expected to be an effective therapy in many pain states and remarkably free of adverse events (26).

HUMAN NEUROPATHIES CAUSED BY GENETIC DEFECTS IN NEUROTROPHINS OR RECEPTORS

Previously, the potential use of neurotrophins was thought to offer symptomatic treatments, as no human degenerative disorder was directly linked to absence or defective production of neurotrophins or mutations in their receptors (87). Recently, however, genetic defects in hereditary sensory and autonomic neuropathies (HSAN), type IV and V, have been identified, resulting from mutations in *TrkA* and *NGFB* genes, respectively (17, 33, 40, 41, 76). These disorders could be the first examples justifying neurotrophic treatment, directed at the underlying etiology.

HSAN IV, also known as congenital insensivity to pain with anhidrosis, is a rare autosomal recessive disorder causing loss of pain sensation and related consequences, such as osteomyelitis and septic arthritis. Most patients also have mental retardation, which is thought to contribute to self-mutilations. Anhidrosis and thermoregulation defects leading to hypothermia, recurrent episodes of unexplained fever and subnormal adrenal function are other well-recognized components of this condition (62). High rates of morbidity and mortality have been found in HSAN IV patients (40). The histopathology is characterized by a complete absence of nonmyelinated and small myelinated axons in the DRG and denervated sweat glands (19).

In the first seven families with HSAN IV defects reported, missense, frameshift, nonsense and splice site mutations were found in an extracellular domain of TrkA that is involved in NGF binding, as well as the intracellular signal transducing domains (41). Mendelian inheritance of the mutations in this autosomal recessive disorder was confirmed in six families for which parent samples were available.

NGF-stimulated autophosphorylation in neuronal and non-neuronal cells were examined by introducing various disease-causing mutations into TrkA cDNA (42). Constructs carrying two mutations in the extracellular domain, L93P and L213P, were expressed in a neuronal cell line, but their products were aberrantly processed and showed diminished NGF-stimulated autophosphorylation in transfected neuronal cells. Five mutations in the tyrosine kinase domain were processed as wild-type TRKA but showed significantly diminished autophosphorylation in both neuronal and non-neuronal cells. This approach detected previously reported double and triple mutations as possible polymorphisms in a particular ethnic background.

HSAN V is a rare autosomal recessive, childhood onset condition associated with loss of temperature control and deep pain leading to ulcers and Charcot joints. Autonomic involvement is variable (62). In contrast to HSAN IV, cognitive functions remained intact in members of the founder family in which a mutation in the coding region of the *NGFB* gene was identified that cosegregated with the disease phenotype (17). This particular mutation in the gene encoding beta subunit of NGF seems to separate the effects of NGF in the development of central nervous system functions (such as mental abilities) from those involved in peripheral pain pathways. In this large consanguineous family from northern Sweden, three severely affected family members were homozygous for a 661C-T transition in the *NGFB* gene. The mutation was predicted to result in a substitution of tryptophan for arginine-211 (R211W), corresponding to position 100 in a highly conserved region of the mature protein (17). Nerve biopsy findings revealed a severe reduction of unmyelinated, and a moderate loss of thinly myelinated, nerve fibers. The identification of mutations in *NGF* and *TRKA* genes illustrates a direct link between this ligand receptor pair and the resulting related disease phenotype.

CLINICAL TRIALS

Diabetic neuropathy. NGF, the most tested growth factor in experimental diabetes models, was the first to be studied in a clinical setting. A three-phase clinical trial was completed in 2000. Phase 1–2 of the rhNGF trial included 250 patients. The study included a placebo group and two experimental groups, which received either 0.1 μg/kg rhNGF or 0.3 μg/kg rhNGF. All subjects received subcutaneous injections three times per week over six consecutive months. The most common side effect was injection site hyperalgesia, which was mild to moderate. Subjects also reported general myalgias. At the study conclusion, global symptom assessment showed a strong beneficial effect of rhNGF treatment on the patients' perception of their neuropathic symptoms, although subjects were partially unblinded because of the injection site hyperalgesia. Both cooling detection threshold and the quantitative neurologic examinations were improved in rhNGF-treated patients compared with the placebo control group. Overall, the study provided preliminary evidence for efficacy in disease treatment, although the authors stipulated that the small size of the study group and brief duration of monitoring may have underestimated the beneficial effects (4).

The subsequent phase 3 trial was a 12-month double-blind and placebo-controlled study with 505 patients with type I or II diabetes mellitus with stable glycemic control. The lower dose from the phase 1–2 trial was selected to minimize injection site discomfort. The treatment group received 0.1 μg/kg of rhNGF by subcutaneous injection three times per week. Patients receiving rhNGF had more secondary adverse effects than the placebo group, including myalgia, peripheral edema and, most often, reported injection site pain/hyperalgesia. Contrary to expectations following the phase 1–2 trial, the phase 3 trial did not demonstrate a therapeutic benefit of rhNGF treatment in diabetic peripheral neuropathy (5). It was argued that the study design was not sensitive enough to detect small-fiber sensory dysfunction, which is the main neuronal population that probably responds to NGF, and that the dose was probably at the threshold of its effective concentration for the disappointing outcome of the phase 3 trial.

A recent double-blind placebo-controlled clinical trial of recombinant human BDNF also did not show any measurable improvement in diabetic neuropathy, although the dosage was both safe and tolerable for patients (94).

Idiopathic painful neuropathy. Idiopathic, painful, small-fiber predominant peripheral neuropathy is resistant to symptomatic treatment. *In vitro*, IGF-1 has been shown to prevent neuronal apoptosis, to increase axonal growth and to support myelination (65). Using a double-blind, placebo-controlled design, 40 patients were randomized for treatment with

recombinant human IGF-1 (0.05 mg/kg twice daily by subcutaneous injection) or placebo for 6 months (95). There were no significant adverse events and the injection site pain occurred equally in both groups. The primary outcome measure was an improved score on an analog pain scale. Secondary end points included quantitative sensory and autonomic testing, neuropathy impairment score, nerve conduction studies, and in the neuropathy symptom and change score. There was no significant difference in the primary end point between the two groups. Analysis of secondary end points and a global impression of improvement by patients and physicians did not show consistent differences between the groups. It was concluded that IGF-1 was safe but did not improve symptoms in this 6-month trial.

Human immunodeficiency virus (HIV) neuropathy. A multicenter, placebo-controlled, randomized phase 2 trial of rhNGF was carried out in HIV-associated sensory neuropathy (45). Sensory neuropathy, which occurs in 30% of individuals with AIDS, is worsened by neurotoxic antiretrovirals. A total of 270 patients were randomized to receive placebo, 0.1 μg/kg rhNGF or 0.3 μg/kg rhNGF by double-blinded subcutaneous injection twice weekly for 18 weeks. The primary outcome was a change in self-reported neuropathic pain intensity. Secondary outcomes included an assessment of global improvement in neuropathy by patients and investigators, neurologic examination, use of prescription analgesics and quantitative sensory testing. In a subset, epidermal nerve fiber densities were determined in punch skin biopsies. Both doses of NGF produced significant improvements in average and maximum daily pain compared with placebo. Positive treatment effects were also observed for global pain assessments ($P = 0.001$) and for pin sensitivity ($P = 0.019$). No treatment differences were found with respect to mood, analgesic use or epidermal nerve fiber densities. Injection site pain was the most frequent adverse event and resulted in unblinding in 39% of subjects. Severe transient myalgic pain occurred in eight patients, usually from accidental overdosing. There were no changes in HIV RNA levels or other laboratory indices. Therefore, a positive effect of rhNGF was found on neuropathic pain and pin sensitivity in HIV-associated sensory neuropathy. It was also concluded that rhNGF was safe and well tolerated, but injection site pain was frequent.

CMT1A. Based on the encouraging results from preclinical studies (see above), a pilot clinical trial was conducted to assess the efficacy of subcutaneously administered recombinant human NT-3 (rhNT-3) in CMT1A patients (72). Eight patients received either placebo ($n = 4$) or 150 mcg/kg NT-3 ($n = 4$) three times a week for 6 months. Myelinated fiber regeneration in sural nerve biopsies before and after treatment served as the primary outcome measure. Additional end-point measures included the Mayo Clinic Neuropathy Impairment Score (NIS), electrophysiological measurements, quantitative muscle testing and pegboard performance. In the treatment group, there was an increase in the mean number of small myelinated fibers within regeneration units ($P = 0.0001$) and also an increase in solitary myelinated fibers, ($P = 0.0002$) in NT-3, not found with the placebo patients. Significant improvements were found in the NT-3 group for NIS ($P = 0.0041$) and trends for improvement were found in the NT-3 group but not in the placebo for the change in the level of discrimination for sensory modalities in the distal limbs for pin, vibration and cold-temperature modalities. Pegboard performance, a measure of neuromuscular function of the hands, was significantly worse in the placebo group. NT-3 did not affect the pegboard performance over the study period. Post-treatment nerve conduction velocities and amplitude of nerve action potentials showed no change from baseline values. NT-3 was well tolerated. In this first pilot clinical study, the findings demonstrating an increase in the mean number of small myelinated fibers within regeneration units and an increase in the thinly myelinated solitary fibers suggest that NT-3 treatment promotes nerve regeneration and may benefit patients clinically, but the results of this study need to be substantiated with a large multicenter clinical trial.

CONCLUSIONS AND FUTURE DIRECTIONS

Research throughout the last half century has produced mounting evidence of the survival and regenerative effects of neurotrophins in the neuronal system. Although early therapeutic trials were not uniformly positive, new insight into the pathogenetic mechanism(s) underlying acquired as well as hereditary peripheral neuropathies calls for new strategies that will realize therapeutic use of neurotrophins in these disorders. In this pursuit, studies detailing the synergistic and opposing effects of neurotrophins and their signaling pathways on axon sprouting and regeneration-associated myelination process in adult nerves in a temporal manner will be important (63, 91, 99). In addition, progress must also be made in alternative methods for more efficacious dose delivery or regulated expression of neurotrophins using viral vectors targeting specific subsets of neurons or SCs at risk (6). Therefore, neurotrophins continue to show promise to be the therapeutic device of the future, not only for peripheral neuropathies but also for other neurodegenerative disorders.

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