Nerve growth factor as potential target in central nervous system disorders: A review

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ABSTRACT

Nerve growth factor (NGF) is a member of neurotrophin family playing important role in growth, development and survival of neurons. This protein is synthesized and released by target tissues and it protects the neurons innervating that tissue from undergoing programmed cell death (apoptosis). NGF exerts its biological action by acting on specific receptor tropomyosin kinase receptor A (TrkA) activating the cytosolic/endosomal pathways which include Ras-mitogen activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K) -Akt, and Phospholipase C (PLC) -γ. NGF interaction with p75 pan-neurotrophin receptor (p75NTR) plays an important role in apoptosis. The function of NGF in neuronal survival and death makes it a potential target in nervous system disorders. NGF is found to play a major role in diseases like Alzheimer’s disease, Parkinson’s disease, amyloid lateral sclerosis, multiple sclerosis and peripheral neuropathies and can have major role in other diseases too. NGF modulator studies are still going on and some drugs are in clinical trials which may produce beneficial outcomes. The current review focuses on the various aspects of NGF as therapeutic tool.

Keywords: Nerve growth Factor, TrkA, p75NTR, Alzheimer’s disease, Parkinson’s disease, amyloid lateral sclerosis, multiple sclerosis, peripheral neuropathies.

INTRODUCTION

Rita Levi Montalcini first introduced the term nerve growth promoting factor in 1954. This factor was first identified as a nucleoprotein which was isolated from mouse sarcoma 180 and 37. This factor was found to have the property of enhancing growth and differentiation in sensory and sympathetic nerve cells. Snake venom and mouse submaxillary glands were later found to be as two potential sources of this factor. The availability of large quantities of the growth-promoting protein extracted from these two sources made possible a better characterization of this factor, so that since 1956 it has been identified as a protein rather than as a nucleoprotein molecule. The biological properties of this factor gave it, its name as the “nerve growth factor” (NGF) [1]. NGF functions as retrograde messenger between target tissues and innervating neurons. NGF is capable of signaling neurons to survive, differentiate or grow. Changes in its levels will affect normal physiology of the Central Nervous System, thus deficiency of nerve growth factor will result in neuronal loss and development of neurodegenerative disorders like Alzheimer’s disease, Parkinson’s disease and other disorders involving neuronal damage. Looking at its neuronal regeneration properties, modulation of nerve growth factor levels can help in treating diseases involving neuronal damage and may help in slowing down progression of disease. Since, the available treatments for neurodegenerative disorders cannot reverse the neuronal damage and NGF being capable of regenerating neurons it is hence being studied for treatment of neurodegenerative disorders like Alzheimer’s disease, Parkinson’s disease etc.

NERVE GROWTH FACTOR:

Nerve growth factor (NGF) belongs to neurotrophin family. It was the first discovered member of the neurotrophin family. Neurotrophins are essential proteins that are required for the survival, growth and functioning of neurons. These belong to a class of growth factors, and function by signaling cells to survive, grow or differentiate progenitor cells to form neurons. These Growth factors are also known as neurotrophic factors. Neurotrophic factors are released by target tissue and act by preventing the associated neuron from initiating programmed cell death - thus allowing...
the neurons to survive. Nerve growth factor is protein and hence gets destroyed in presence of proteolytic enzymes (trypsin, pepsin, and chymotrypsin) [2]. Members of neurotrophin family includes Nerve growth factor (NGF) which binds to TrkA, Brain-derived neurotrophic factor (BDNF) which binds to TrkB, Neurotrophin-3 (NT-3) which binds to TrkC, Neurotrophin-4/5 (NT-4/5) binds to TrkB [3]. NGF supports the survival and differentiation of sensory neurons, induces neural processes, and regulates the expression of neurotransmitters and the connection between the neuron and the target [4].

**Structure:**

![Diagram of Nerve Growth Factor (NGF) binding to p75NTR](image)

*Fig 1: Binding of Nerve growth factor to p75* 
Nerve growth factor is a protein. NGF is produced as a precursor complex of about 130 kDa (7S-NGF). This complex comprises of alpha, beta and gamma subunits. Alpha and gamma subunits are members of the kallikrein family of serine proteinases and are involved in the cleavage of the 7S-complex and releasing beta-NGF.

**Biological sources**

- The synthesis of Nerve Growth Factor in adult tissues depends on PNS neuronal cell phenotypic features, such as their innervation density, size, axonal terminal sprouting, dendritic ramification, induction or inhibition of neuropeptides and neurotransmitters or transmitter producing enzymes. In the central nervous system (CNS), the greatest amount of this neurotrophin is produced in the cortex, hippocampus and pituitary gland; although significant quantities of nerve growth factor are also produced in areas like basal ganglia, thalamus, spinal cord and retina [2].
- The NGF molecule is a normal constituent of the embryonic sensory and mature sympathetic nerve cells, and it is also present in many organs and tissue extracts; it is found in trace amounts in the serum of all mammals [2].
- The mature, active form of NGF descend from proteolytic cleavage of a precursor form (ProNGF), that have important roles during development as well as in adult life [2]. Mature neurotrophin arise from the proteolytic cleavage of the precursor form by various proteases like plasmin, furin, MP7, PC1/3, PC2, PC5/6, PC7 and PACE4 [5].
- The basal keratinocyte also synthesize and secrete NGF in the human adult epidermis. The paracrine secretion of NGF by keratinocytes might have a major role in regulating innervation, lymphocyte function, and melanocyte growth and differentiation of epidermal cells as well as during wound healing [6].

Nerve growth factor is also produced by other organisms which can be used for its therapeutic effects some of the sources are as follows:

**NGF from Mouse Submaxillary Salivary Glands:** The mouse submaxillary salivary glands secrete NGF from puberty and levels of it increases in subsequent developmental stages to reach a plateau in the adult male. It’s concentration is 10 times higher in male than in female glands. Injections of testosterone result in a marked increase in the NGF content in the female gland, whereas castration sharply decreases the content of the same protein in males. The NGF is synthesized and stored in cells of the tubular portion of this gland [1].

**NGF from Snake Venom:** NGF was detected in three families of poisonous snakes: Elapidae, Viperidae, and Crotalidae. The majority of snake venom NGFs are non-covalently bound dimers with molecular mass of about 25 kDa containing two identical (or very similar) subunits with molecular mass of about 13 kDa. NGFs from *Bitis arietans* and *Bungarus multicinctus* are covalently bound dimers. Several venom NGFs have been identified as glycoproteins: *Bothrops atrox*, *Vipera russelli*, *Vipera lebetina* nerve growth factors contain carbohydrates. At present, many NGFs have been cloned and sequenced and their sequences are highly homologous. Two cDNAs encoding isoforms of NGF have been isolated from *Naja sputatrix*, and purified NGF have been obtained from *N. sputatrix* venom [7].
Functions of nerve growth factor

- NGF is required for its survival effects, differentiation and maintainence function of sympathetic and embryonic sensory neurons. This protein has striking growth effects on sensory and sympathetic nerve cells, but only sympathetic nerve cells remain receptive to its action throughout their life cycle. Nerve growth factor also induces differentiation of progenitor cells, to form neurons [2].
- NGF is essential for the growth and maintenance of neurons in the peripheral nervous system (PNS) and for the functional integrity of cholinergic neurons in the central nervous system (CNS) [2].
- In the dorsal root ganglion (DRG) the expression of neuropeptides such as Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) by primary sensory neurons is under NGF control and in vivo deprivation of NGF, as a result of nerve transection or anti-NGF treatment, causes a marked decrease in SP and CGRP synthesis [2].
- NGF has effect on neuronal plasticity that allows the adult nervous system to modify its structure and functions in response to stimuli [2].
- The NGF plays an important role in the survival and function of cholinergic neurons of the basal forebrain, they play an important role in attention, motivation, arousal, memory and consciousness. In the CNS, NGF regulates phenotypic features in noradrenergic nuclei of hypothalamus and brainstem [2].
- NGF is essential for the survival and differentiation and maintainence of phenotypic features of granulocytes, lymphocytes, monocytes and hematopoietic stem cells [2].
- NGF enhances T and B-cell mediated immune responses, induces human B and T-lymphocyte differentiation and proliferation, influences shape changes of platelets, accelerates wound healing, and promotes growth, survival and functional properties of neutrophils like chemotaxis, phagocytosis and superoxide production and acts as an autocrine survival factor for memory B-lymphocytes [8].
- The production of norepinephrine in sympathetic neurons is under control of NGF. It acts by selective induction of tyrosine hydroxylase (TH) [2].
- NGF also has effects on mast cells. NGF receptors are found in immune organs and on immune cells allowing NGF to modulate cell differentiation and regulate the immune response. NGF concentrations in the tissues change during inflammation and inflammatory mediators induce NGF synthesis in a variety of cell types. Also, production of NGF increases in inflamed tissues of patients with inflammatory and autoimmune diseases [4].

MECHANISM OF ACTION:

Fig 2: Binding of NGF & proNGF
NGF exerts its biological action by acting on specific receptor tropomyosin kinase receptor A (TrkA), which is a typical tyrosine kinase receptor (Fig 1 & 2). NGF molecules occur in pairs, but they do not bind to a pair of receptors. But when two NGF molecules bind to a single p75 receptor, there binding produces a conformational change. The cytosolic/endosomal pathways activated by the TrkA are Ras-mitogen activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K) -Akt, and Phospholipase C (PLC) –γ. NGF can bind to and activate the low-affinity and non-selective p75 pan-neurotrophin receptor (p75NTR). p75NTR receptor is a transmembrane glycoprotein that regulates signaling through TrkA; binding of NGF to p75NTR activates additional signaling pathways that, in the absence of co-expressed TrkA, may signal a cell to die via apoptosis. Signaling pathways activated by p75NTR are the Jun kinase signaling cascade, NF-κB and ceramide generation. Binding of proNGF to p75NTR also leads to apoptosis. p75NTR activation can lead to cell apoptosis if this effect is not counteracted by anti-apoptotic signal generated by activation of TrkA (Fig 3) [2].

**Fig 3: Activation pathway of TrkA by Nerve growth factor (NGF)**

NGF binds to the high-affinity tyrosine receptor TrkA, activating several signaling pathways which have effect on both morphological and transcriptional targets. The signaling molecules like PLC-γ, PI3K, MAPK, and JNK, are activated upon the addition of NGF. PLC-γ brings about the hydrolysis of phosphatidylinositol-4, 5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol triphosphate (IP3). The resulting DAG formed activates protein kinase C whereas IP3 promotes transient release of Ca2+ from the Endoplasmic reticulum. The activation of pathway including PLC-γ is responsible for neuronal cell differentiation and neurite outgrowth [9].

**NERVE GROWTH FACTOR AS POTENTIAL TARGET IN CNS DISORDERS**

**Alzheimer’s Disease:** Alzheimer’s is a disease affecting memory, thinking and behavior of an individual. Memory related changes occurring in Alzheimer’s disease are not a normal part of aging. Alzheimer’s is a disease which worsens with time. Symptoms can vary with individuals but the first problem many people notice is forgetfulness severe enough to affect their ability to function at home or at work. The disease may cause a person to become confused, forget familiar places, misplace things or can cause trouble with language.

Signs of Alzheimer’s/dementia
- Lack of judgment and decision making
- Inability to manage things
- Difficulty in conversation
- Inability to remember things
Alzheimer’s disease (AD) is a cognitive disorder that currently affects about 18 million people worldwide. The risk of developing AD increases with increasing age. The cause of the AD pathology is an abnormal deposition of the beta amyloid peptide. Nerve growth factor (NGF) protein plays an important role in the maintenance of the basal forebrain cholinergic neurons (BFCNs). Cholinergic neurons in basal forebrain are highly affected in Alzheimer’s disease hence, NGF can serve as a potential protective and/or curative factor for neurodegenerative disorders associated with these neurons [2]. NGF is released as a precursor molecule, which is converted to its mature form (mNGF) and then degraded by activated metalloprotease 9 (MMP9). In normal brains, proNGF is released to the extracellular space by cortical pyramidal neurons along with the convertases (tPA, MMP-9) and zymogens (plasminogen) necessary for its processing to mature NGF (mNGF) and its degradation. The NGF molecules that are not taken back to the basal forebrain by projecting cholinergic neurons (BCFN) are degraded by MMP-9. Maintenance of BFCN phenotype is dependent on continuous supply of NGF. In AD, the lack of trophic support to BFCN can be partly explained by dysregulation in the expression and activity of the members of the NGF metabolic pathway. The presence of intercellular Abeta oligomers initiates a pro inflammatory reaction that affects the regulation of the NGF metabolic pathway. There is fall in Plasminogen, tPA and plasmin levels which explains the paradoxical elevation of the NGF precursor in the presence of BCFN atrophy in AD. In addition, more of the endogenous NGF degrades, as MMP-9 levels and activity are highly increased. Cholinergic neuron loss is a major clinical feature of Alzheimer disease. Nerve growth factor (NGF) stimulates cholinergic function, improves memory and prevents cholinergic degeneration in amyloid overexpression and aging. The majority of acetylcholine is released by Basal forebrain cholinergic neurons in the cerebral cortex and hippocampus, enhancing synaptic efficacy and modulating active cortical circuits. These Basal forebrain cholinergic neurons degenerate in Alzheimer disease, resulting in cognitive decline. NGF does not cross the blood brain barrier when administered peripherally because of its size and polarity, when infused into the brain ventricular system. NGF causes side effects from its broad distribution, including pain (from stimulating dorsal root ganglia nociceptive neurons), weight loss (from hypothalamic stimulation) and Schwann cell migration into the spinal cord and medulla. For clinical use, NGF administration must therefore meet two requirements: it must be delivered in needed quantities to stimulate neurons, and its distribution must be localised to areas of degenerating neurons to avoid adverse effects. Gene delivery meets these requirements. Using either ex vivo (genetic modification of cells in vitro) or in vivo (genetic modification of cells in the brain itself) gene therapy, such growth factors can be delivered directly to the brain and can diffuse for distances of 2–5 mm². Autologous fibroblasts which were genetically modified are able to produce and secrete human NGF. They survive grafting to the brain and sustain NGF production for at least 18 months, preventing cholinergic degeneration, improve cholinergic neuron function and memory, implanted cells are safe, they are not tumorous, nor they migrate and cause no discernable toxicities in dose-escalation studies. This procedure is used in humans here, autologous fibroblasts are removed from small skin biopsies in each subject and were genetically modified to
produce and secrete human NGF using retroviral vectors. NGF production was measured, and cells were stereotaxically injected into the cholinergic basal forebrain (a region ~1 cm in length) in one surgical session. Cognition was measured on a second scale commonly used in Alzheimer disease trials, the Alzheimer Disease Assessment Scale-Cognitive subcomponent (ADAS-Cog). Decrease in cognitive decline was observed in patients who received this therapy. The patients were not taking any other medications during this trial [10]. Thus, it was found from above study that improving NGF production in areas of neuronal damage was able to protect these neurons from further degeneration and improving their function. Clinical trials are going on to test efficacy of preparations containing NGF.

**Drug:**
CERE-110 is the preparation which carries the gene encoding NGF encased in a harmless viral coating (adeno-associated virus) that protects the gene and facilitates its delivery to brain cells. CERE-110 in phase 1 trial showed that it is well tolerated [11]. CERE-110 is in phase 2 trial [12].

**Parkinson’s disease**
It is a progressive disorder of the Central Nervous System that typically affects its victims around age 60. Neurons in the area from the substantia nigra to the putamen and caudate nucleus degenerates in PD these neurons release the neurotransmitter dopamine (DA) [13].

- **Signs of Parkinson’s disease**
  - Tremor (shaking)
  - Slowness of movement
  - Rigidity (stiffness)
  - Difficulty with balance

**NERVE GROWTH FACTOR IN PARKINSON’S DISEASE**
Parkinson’s disease is a progressive disorder of the CNS that typically affects its victims around age 60. Neurons that extend from the substantia nigra to the putamen and caudate nucleus degenerate in PD these neurons release the neurotransmitter dopamine (DA) [13].

Nerve growth factor increases the survival, neurite outgrowth, and functional effect of grafts of adrenal chromaffin cells to the basal ganglia. Implantation of adrenal tissue pieces produced a transient functional improvement which can last for a few months. Grafting of adrenal chromaffin tissue into the putamen was done, which was then supported by infusion of nerve growth factor. Through the cannula, nerve growth factor can be infused. This method decreased rigidity & hyperkinesia. It seems, however, that nerve growth factor treatment may be safe in human beings and that it may prolong the effect of adrenal chromaffin grafts [14].

**Amyotrophic lateral sclerosis (ALS)**
ALS is a neurodegenerative disorder. ALS is motor neuron disease (MND). ALS causes muscle spasticity and progressive weakness due to muscle wasting. Muscle weakness makes it difficult for ALS patients to speak, swallow and breathe. The patients die from respiratory failure within 3 years after first symptom is observed. The risk of developing this disease is more between ages of 50 and 75 years [16].

- **Signs of Amyotrophic lateral sclerosis**
  - Difficulty walking, tripping or difficulty in doing normal daily activities
  - Weakness in leg, feet or ankles
  - Hand weakness or clumsiness
  - Slurring of speech
  - Trouble swallowing
  - Muscle cramps, twitching in arms, shoulders and tongue

**NERVE GROWTH FACTOR IN AMYTOTROPIC LATERAL SCLEROSIS**
Amyotrophic Lateral Sclerosis, or ALS, is a serious neurological disease that affects the ability to move. It is also called Lou Gehrig’s disease. ALS attacks the neurons that control muscles, the motor neurons. Messages from motor neurons in the brain called upper motor neurons are transmitted to
motor neurons in the spinal cord called lower motor neurons, then passed on to the muscles. With ALS, both the upper motor neurons and the lower motor neurons die and stop sending messages to muscles. Here, only motor neurons are affected [17].

Nerve growth factor (NGF) plays an important role in neuronal survival and differentiation through activation of the tyrosine kinase receptor TrkA, but it can also stimulate neuronal death by activation of p75NTR. In amyotrophic lateral sclerosis (ALS) patients and in animal models there was overexpression of ALS-linked mutant superoxide dismutase (SOD) and degenerating motor neurons were surrounded by reactive astrocytes and NGF levels were increased in degenerating lateral column of the spinal cord. Motor neurons lack TrkA, which is the only Trk receptor responsive to NGF. Motor neurons express p75NTR during the embryonic period of naturally occurring cell death but expression gradually ends after birth. Although adult motor neurons do not express p75NTR, but is found in motor neurons of ALS patients. NGF produced endogenously by reactive astrocytes in ALS is responsible for apoptosis in p75NTR expressing motor neurons. These astrocytes caused damage to motor neurons further causes p75NTR induction. The damage to these neurons is permanent. ALS is fatal. Treatment includes treating symptoms and on average patients die within three to four years after first appearance of symptoms [17].

In Amyotrophic Lateral Sclerosis, NGF levels increase but motor neurons lack TrkA to which NGF binds to produce its neuronal survival effects. Instead, motor neurons of ALS patients show presence of p75NTR receptors which on activation by NGF in absence of TrkA activation causes death of motor neurons. Antibodies that block p75NTR and NGF were found to decrease the motor neuron death in cell culture studies. Further studies are still going on.

**Multiple sclerosis:** It is an autoimmune disease that causes a progressive destruction of myelin sheaths surrounding neurons in the CNS. It usually appears between ages of 20 and 40, affecting females twice as often as males. It is less common in Asians [13].

**NERVE GROWTH FACTOR in multiple sclerosis:** Multiple sclerosis is immune mediated condition of the central nervous system in which myelin sheath of nerve fibres is destroyed. Neurotrophic factors belong to family of proteins which includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and others, that are thought to play a role in preventing neural death and in favoring the recovery process, neural regeneration, and remyelination. Multiple sclerosis (MS) is an inflammatory and degenerative disease, characterized by different patterns of demyelination and axonal loss. In experimental study, NGF was found to bring about myelin repair. There was induction of neurotrophic factors under acute exercise which was observed in study in healthy controls as well as MS patients which may potentially be involved in beneficial effects of aerobic training [18, 19]. Intracerebroventricular (ICV) infusions of recombinant human NGF in non-human primates showed decrease in demyelination. Studies are still going on to observe benefits of nerve growth factor in treatment of multiple sclerosis. In Multiple sclerosis there is neuronal damage due to hyperactivity of immune system and neuronal damage is found to be reversed when supplied with NGF. Clinical trials involving gene therapy of NGF to repair and protect neurons from further damage are still going on.

**OTHER CLINICAL USE OF NGF**

**Diabetes and peripheral neuropathies:** Diabetes is often characterized by major complications such as dysfunction and degeneration of several types of PNS neurons/fibers. Sensory neuronal involvement is predominant, the sensory fiber degeneration being responsible for the more debilitating symptoms. Deficits in NGF transport, serum and tissue content have been demonstrated in experimental diabetes. Major components of the NGF signaling pathway have been also found to be affected in experimental diabetes, as well as the production of neuromodulators that is known to be under NGF control was found to be affected. When NGF was supplied in animal models of diabetic neuropathies it was able to reverse neuropathic signs, by protecting the affected neurons and normalizing their function. The production of recombinant human NGF (rhNGF) has been developed and tested in phase I clinical trial, side effects, such as myalgias and injection site hyperalgesia, were observed in healthy subjects. A phase II clinical trial was conducted on 250 patients with diabetic polyneuropathy. It was observed that there was improvement in neuropathic symptoms in patients treated with NGF, but side effects, such as hyperalgesia, myalgias and arthralgias at injection site were also observed [2].

**Peripheral neuropathies:** NGF is produced and released by tissues, which then binds to specific receptors (TrkA) on nerve terminals to exert its neurotrophic activity. Changes in the neurotrophic circuit can cause peripheral nerve to lose its normal function. Data from animal models and human
pathologies showed that disease-associated peripheral neuropathies could be either due to deregulation of NGF synthesis, transport and utilization by PNS neurons. This makes NGF an important factor in the development of neuropathic symptoms occurring in diabetes, HIV infections or chemotherapy, and suggests neurotrophin as a potential pharmacological tool in the treatment of peripheral neuropathies [2].

**Human immunodeficiency virus:** Peripheral nerve damage in human immunodeficiency virus (HIV) patients can be caused by virus itself or from the anti-viral drugs. A phase II clinical trial with subcutaneous recombinant human nerve growth factor (rhNGF) was performed on 270 HIV-infected patients with sensory neuropathy a decade ago. From the study, rhNGF was found to be effective in neuropathic pain, but with side effects like pain at injection site. From the study, rhNGF was found to be safe and well tolerated. When long term (48 weeks) effect of rhNGF was studied in 200 subjects with HIV related distal sensory neuropathy, it was observed that even after being safe and well tolerated NGF did not show any improvement in severity of neuropathy which was determined by epidermal nerve fiber density, neurological examination and quantitative sensory testing [2].

**CONCLUSION:**

Current treatment for Neurodegenerative disorders is limited to preventing the progress of the disorders &/or curing the symptoms. The need of newer approaches is to reverse the neurodegenerative diseases. Nerve growth factor is important for survival of neurons and has been found to play important role in neurodegenerative disorders. Nerve growth factor is one of such upcoming molecule which can be useful to reverse the neurodegenerative disease. Strategies that aim at correcting deficits in NGF metabolism will have potential therapeutic benefit for cholinergic neuroprotection in subjects with Alzheimer’s disease. In a similar way therapeutic benefits in Parkinson’s disease, multiple sclerosis & other such neurodegenerative disorders can also be observed in near future using nerve growth factor. This molecule can be further explored and studied for its therapeutic uses in treating the central nervous system disorders.

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