Brain Targeting Of Nerve Growth Factor Using Poly(Butylcyanoacrylate) Nanoparticles

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Citation

Abstract
Nerve growth factor (NGF) is essential for the survival of both peripheral ganglion cells and central cholinergic neurons of the basal forebrain. The accelerated loss of central cholinergic neurons during Alzheimer's disease may be a determinant of dementia and this may suggest a possible therapeutic benefit for NGF. However, NGF as all other neurotrophic factors does not significantly penetrate the blood brain barrier, the fact that makes its clinical usefulness depends on the use of suitable carrier system enhances its transport through BBB. The present investigation examines brain delivery of nerve growth factor adsorbed on poly(butylcyanoacrylate) nanoparticles coated with polysorbate-80 and testing its effectiveness in reversing of scopolamine-induced amnesia in model of acute amnesia in rats using the passive avoidance reflex (PAR) test. Systemic administration of NGF adsorbed on PBCA-nanoparticles coated with polysorbate-80 successfully reversed scopolamine-induced amnesia and improved recognition and memory in acute amnesia rat model. This appeared in form of significant increase in the mean latent period of (PAR) test in the group of animals treated with NGF adsorbed on PBCA-nanoparticles coated with polysorbate-80 compared with the group treated with free NGF. In summary, these results demonstrate that, using poly(butylcyanoacrylate) nanoparticles coated with polysorbate-80 as a carrier system is effective in targeting NGF to the central nervous system and may optimize the therapy of age-related neurodegenerative diseases.

INTRODUCTION
Nerve growth factor (NGF) which was discovered in the early 1950s, when it was found that; mouse sarcoma tissue transplants in chicken embryos caused an increase in the size of spinal ganglia, was the prototypical neurotrophic factor for many decades (1). It represents the most known and studied trophic factor, which acts on sensory and sympathetic neurons of the peripheral nervous system, and on basal forebrain and striatal cholinergic neurons of the central nervous system (2). The specificity and trophic actions of NGF on these neuronal populations and its efficacy in preventing neurodegeneration have led to its proposal for evaluation in the treatment of neurological diseases such as Alzheimer’s disease, diabetic neuropathies and Huntington’s disease. Preclinical and clinical studies carried out on animal models and patients with diagnosis of these diseases have revealed satisfactory results (3). The cloning studies demonstrated that; NGF is a member of a gene family dubbed neurotrophins, comprising in mammals also NT-3 and NT-4/5. Currently, six neurotrophins have been isolated: NGF, BDNF, NT-3, NT-4 (also known as NT-5), NT-6, and NT-7. There is substantial evidence that they all arose through successive duplications of the genome of an ancestral chordate (4). The chemical structure of NGF was well studied. It consists of 3 types of subunits, alpha, beta and gamma, which specifically interact to form a 7S, 130,000-molecular weight complex. This complex contains 2 identical 118-amino acid beta-chains, which are solely responsible for nerve growth stimulating activity of NGF (5). NGF is synthesized from a precursor peptide “proneuropin” which has low biological activity compared with the parent members of neurotropins, and is activated by peptide cleavage via enzymatic processes occur at the site of its synthesis (6).

After release in target areas of NGF-responsive neurons, nerve growth factor binds to specific cell-surface receptors on the nerve terminals and is retrogradely transported to the cell bodies (7). NGF mediates its cellular responses through binding to two distinct types of cell surface receptors: the high affinity (Trk NTR), family of receptor tyrosine kinases, which exhibit high selectivity for neurotrophin binding, and the low-affinity (p75 NTR) neurotrophin receptor, a member of the tumor necrosis factor receptor family that binds all neurotrophins with similar affinity (p75). Trk receptors are members of the family of tyrosine kinase receptors which
have high affinity for neurotrophins and different specificities for different members of the neurotrophin family. The known parts of the extracellular domain contain three leucine-rich repeats and two fibronectin-III modules. The intracellular kinase domain is the same of insulin-receptor tyrosine kinase (1). The Trk proto-oncogene family contains four members, TrkA, TrkB, TrkC, and TrkE which are variably expressed throughout the central and peripheral nervous systems. Individual neurotrophins activate different Trk receptors (NGF acting at TrkA, BDNF and NT-4/5 act at TrkB, and NT-3 predominantly, but not exclusively, at TrkC) (1). Binding of the neurotrophins to the Trk receptors leads to receptor tyrosine phosphorylation (1). This triggers the activation of pathways leading to the prevention of programmed cell death and neuronal differentiation. Neurotrophin p75 receptors are transmembrane glycoprotein receptors of ~75 KD. They are members of the tumor necrosis factor receptor family that have low-affinity to neurotrophins and bind with all neurotrophins with similar affinity but with different kinetics. In vitro, unbound p75 receptors have been shown to promote neuronal cell death, whereas binding of p75 by NGF ligand or antibody has been shown to inhibit p75-induced cell death. P75 has been immunohistochemically demonstrated in neuronal axons, schwann cells and perinural cells of peripheral nerves (1). Although TrkA is able to elicit responses of neurotrophins by itself in some systems, p75 can co-operate with TrkA to increase its binding to neurotrophins and activation of TrkA at low ligand concentration (1).

NGF and other neurotrophic factors are essential for the development and maintenance of the nervous system. They play a major role in regulating growth, survival, and differentiation of neuronal cells (1). They enhance the secretion of neurotransmitters as reflected by an increase in the frequency of miniature synaptic events both at frog neuromuscular junctions or synapses of cultured hippocampal neurons (1). Also, nerve growth factor and BDNF have been reported to augment the evoked release of acetylcholine and glutamate from hippocampal synaptosomes. In addition to these results, neurotrophins induced long lasting potentiation of neurotransmission or enhanced long term potentiation in hippocampal slices (1). They support the survival of specific types of neurons and neurotransmitter systems, and are synthesized and secreted by cells that are the targets of specific innervating neurons (1). NGF plays a role in the maintenance of the cholinergic neurotransmitter system in specific populations of neurons, including a group of cholinergic forebrain neurons (1). Nerve growth factor (NGF) is the most potent growth stimulating factor for cholinergic neurons and therefore is a promising candidate for treating Alzheimer’s disease, however, the delivery of NGF to the brain must the solved (1). NGF as other neurotrophic factors is hydrophilic, typically basic compound, the blood-brain and blood-cerebrospinal fluid barriers severely limit its ability to enter into and act on its target sites in the CNS following parenteral systemic administration. Recently, many studies tried to describe a useful drug delivery system for transport of NGF across the BBB to the CNS such as pegylation technology which involves conjugation of NGF with polyethylene glycol (PEG-2000) (1), covalently conjugation to anti-transferrin receptor antibodies (OX26 murine mAb) by the avidin/biotin technology, (1,2), and adsorption on liposomes (1).

These are promising tools for clinical use of neurotrophic factors but their investigational studies are still in the beginning. Also, recent studies have shown that drugs that are normally unable to cross the blood brain barrier following systemic administration can be transported across this barrier by adsorbing such drugs on poly(butyclyanoacrylate) nanoparticles and coating with polysorbate-80. Drugs that have successfully been transported into the brain using this carrier include the hexapeptide dalargin (1,2,3), the dipeptide kytorphin (1), loperamide (1), tubocurarine (1), the NMDA receptor antagonist MRZ 2/576 (1), and doxorubicin (1). The possibility of drug transport into the brain by nanoparticles opens up totally new perspectives for the treatment of CNS diseases and studying its use in transporting of neurotrophic factors. The aim of this investigation is to study the brain delivery of nerve growth factor using poly(butyclyanoacrylate) nanoparticles coated with polysorbate-80 as “Torjan horse” carrier system.

**MATERIAL AND METHODS**

**ANIMALS**

All experiments were carried out on Albino male rats of body weights (250-300 g). The animals were kept in groups in plastic cages 7-10 rats in each, at room temperature 18-20 C° in 12 hours light cycle/day with free access to water and food ad libitum. To minimize the possible bias of circadian rhythm all drug injections were done in the morning.
DRUG SUBSTANCES AND PREPARATION METHODS OF DRUG FORMS

In the experiments the following substances were used:

Scopolamine (powder), 7S Nerve growth factor, Mouse submaxillary glands, lyophilized (CALBIOCHEM®, Germany). Poly(butylcyanoacrylate) nanoparticles, lyophilized powder in vials, 1.8 gm in each vial (prepared in the department of molecular chemistry of institute of a molecular pathology of Russian Academy of Medical Science).

PREPARATION OF WORKING SOLUTIONS

Scopolamine was dissolved in isotonic sodium chloride solution in a concentration gives a dose of 2 mg/kg in 1 ml.

Nerve growth factor was dissolved in isotonic sodium chloride solution in a concentration gives a dose of 5 µg/rat in 0.1 ml.

Solutions of nerve growth factor with polysorbate-80

For preparation of a mixture of solutions of nerve growth factor with polysorbate-80, the working solution were prepared (as shown above), then to this solution polysorbate-80 1 % solution is added in a rate 1 µL polysorbate-80 to 1 ml working solution of nerve growth factor.

PREPARATION SUSPENSION OF NANOPARTICLES ADSORBING NERVE GROWTH FACTOR (NGF) AND COATED WITH POLYSORBATE-80

The lyophilized powder of poly(butylcyanoacrylate) (1.8 gm) was dispersed in 0.8 ml isotonic solution of sodium chloride to obtain homogenous suspension with milky white color.

Lyophilized powder of nerve growth factor (45 µgm) dissolved in 1 ml of isotonic sodium chloride solution.

Solution of NGF is mixed with nanoparticles suspension and the mixture was incubated at room temperature for 3 hours. On this suspension, 1 % solution of polysorbate-80 was added and the mixture was incubated for more 30 minutes. The result were a 1.8 ml 1 % suspension poly(butylcyanoacrylate) nanoparticles contain 45 µgm NGF (5 µgm NGF in 0.2 ml suspension).

THE PASSIVE AVOIDANCE REFLEX TEST

(PAR)

APPARATUS

The passive avoidance reflex test (PAR) was carried out using “Lafayette instrument - USA” apparatus. The apparatus consists of two compartments, metallic lighted platform (25x7 cm) connected to a black chamber (40x40x40 cm). Between the platform and the chamber there is a door through which the animal freely can move from the platform to the chamber. The floor of the chamber consists of a metal grid through which electric painful stimuli are induced through the paws of animals (5 shocks, 1 mA, 1 Sec. duration. for each with 2 sec. intervals).

PASSIVE AVOIDANCE PARADIGM

A one-trail step-through passive avoidance task was carried out on the apparatus above described. The door between the platform and black chamber was opened. Rat was placed on the lighted platform in a position its tail directed to the door. Trying to leave the annoying bright light, the rat entered the black chamber through the door. The time, in seconds, spent by the rat before entrance in the dark chamber (latent period) is measured. Once the rat enters, the door is blocked and the animal receives a series of electric stimuli (5 shocks, 1 mA, 1 Sec. duration. for each with 2 sec. intervals). This represents the training part of the test. Within 24 hours, the results of training are estimated. For this purpose, rat again is placed on the lighted platform and the latent period of entrance of animal in the dark chamber is measured while the electro-painful stimuli are not rendered.

MODEL OF ACUTE AMNESIA INDUCED BY SCOPOLAMINE

The experiments represent a slight modification of the method described by Petkov et al., 1988. In this method, model of acute amnesia in rats is induced by subcutaneous injection of rats with scopolamine in a dose 2 mg/kg 30 minutes before training in the step-through passive avoidance reflex test (PAR) to determine the memory changes. Tested drug forms were injected immediately after injection of scopolamine. Test for measurement of latent period in PAR test was carried out within 24 hours after training.

STATISTICS

Statistical treatment of data was carried out using “Sigma-plot” computer program where unpaired student-t test was used to test significance of the differences between means.
RESULTS

60 male rats were used, divided into 6 groups (10 rats in each group). Groups were treated with the following substances combined with the following drug forms:

Group 1: 0.9 % NaCL solution intrapronely (passive control).
Group 2: scopolamine (2 mg/kg/s.c.) (active control).
Group 3: scopolamine (2 mg/kg/s.c.) and NGF (5 µg/rat, i.p).
Group 4: scopolamine (2 mg/kg/s.c.) and NGF (5 µg/rat mixed with 1 % polysorbate-80 solution, i.p).
Group 5: scopolamine (2 mg/kg/s.c.) and NGF (5 µg/rat, adsorbed on nanoparticles, i.p).
Group 6: scopolamine (2 mg/kg/s.c.) and NGF (5 µg/rat, adsorbed on nanoparticles coated with polysorbate-80, i.p).

Results are shown in figures 1, 2 and 3

Figure 1

Figure 1: Effect of NGF alone (Group 3), NGF with polysorbate-80 (Group 4), NGF adsorbed on PBCA-nanoparticles (Group 5) and NGF adsorbed on PBCA-nanoparticles coated with polysorbate-80 (Group 6) on the latent period of scopolamine-induced amnesia in passive avoidance reflex (PAR) test in rats. Group 1 saline treated (passive control) group. * Significant difference (p < 0.05) from saline group (Group 1). ** Significant difference (p < 0.01) from saline group (Group 1).

Figure 2

Figure 2: Effect of NGF alone (Group 3), NGF with polysorbate-80 (Group 4) and NGF adsorbed on PBCA-nanoparticles (Group 5) and NGF adsorbed on PBCA-nanoparticles coated with polysorbate-80 (Group 6) on the latent period of scopolamine-induced amnesia in passive avoidance reflex (PAR) test in rats. ** Significant difference (p < 0.01) from scopolamine-treated group (Group 2).

Figure 3

Figure 3: Effect of NGF alone (Group 3), NGF with polysorbate-80 (Group 4), NGF adsorbed on PBCA-nanoparticles (Group 5) and NGF adsorbed on PBCA-nanoparticles coated with polysorbate-80 (Group 6) on the latent period of scopolamine-induced amnesia in passive avoidance reflex test in rats. * Significant difference (p > 0.05) from NGF-treated group (Group 3). ** Significant difference (p < 0.01) from NGF-treated group (Group 3).

In group 1 treated with 0.9 % NaCL solution (passive control), the mean latent period of entrance in the black
chamber in PAR test was 113.9 second.

In group 2 treated with scopolamine (active control), the mean latent period recorded was significantly decreased to 47.5 second, that indicated presence of amnesic effect.

In group 3 treated with NGF after scopolamine injection, the mean of the recorded latent periods was 84.8 second, this value is intermediate between the mean latent periods of both active and passive control groups, but statistically insignificant compared with active-control group.

In group 4 treated with NGF mixed with polysorbate-80 after scopolamine injection, the mean of the recorded latent periods was 127.4 second that indicated significant increase (p ≤ 0.01) in latent period compared with the active control group and significant increase (p ≤ 0.05) compared with group-3 treated with free NGF.

In group 5 treated with NGF adsorbed on nanoparticles after scopolamine injection, the mean of the recorded latent periods was 124.8 second. This represents significant increase (p ≤ 0.01) in latent period relative to active control group and significant increase (p ≤ 0.05) compared with group-3 treated with free NGF.

In group 6 treated with NGF adsorbed on nanoparticles and coated with polysorbate-80 after scopolamine injection, the mean of the recorded latent periods was 178.3 second. This represented the greatest increase in the latent period compared with both active and passive control groups and also significant increase (p ≤ 0.01) compared with group-3 treated with free NGF. This indicates that NGF adsorbed on nanoparticles and coated with polysorbate-80 not only reverse scopolamine induced amnesia but also enhanced the cognitive functions of animals, raising the training results in PAR test.

DISCUSSION

When free nerve growth factor (NGF) was administered systemically after induction of amnesia by scopolamine injection, it did not produce any significant change in the mental or cognitive activity of animals which was represented by the change in the latent period in the passive avoidance reflex test. These results are in accordance with the results obtained by (40) that indicated that after intravenous administration of NGF, it exerts no any central effects while, after its intracerebroventricular injection, it has a nootropic activity and improves the growth and function of central cholinergic neurons. Administration of NGF with polysorbate-80 significantly increased the latent periods recorded in acute model of amnesia in the passive-avoidance reflex test compared with scopolamine-treated group. This reflects the improvement in the cognitive activity impaired by scopolamine, which indicates that, polysorbate-80 alone, is able to enhance the penetration of peptides as NGF through BBB hence; NGF started to reach a suitable concentration in the CNS and started to exert a measurable pharmacological effect. NGF adsorbed on PBC-nanoparticles and coated with polysorbate-80 when administered in scopolamine-treated group led to statistically significant increase in the latent period of passive avoidance test of rats compared with scopolamine-treated group and statistically significant increase (p ≤ 0.01) in latent period compared with group treated with NGF alone. This can be attributed to the ability of polysorbate-80 coated nanoparticles to enhance the penetration of NGF through the BBB and increase the available amount of NGF at its target site of action. These findings can be explained as follows: NGF is well known to enhance neuronal activity particularly the central cholinergic neurons. It was reported that, it has the ability to prevent cholinergic neuronal atrophy in addition to its ability to enhance central cholinergic transmission in hippocampal neurons via its action on postsynaptic tyrosine kinase receptors (37). Also, it was reported that, cholinergic neurons are NGF-sensitive and NGF-dependent and this represents the base for the ability of NGF to improve cognitive functions and disorders in which central cholinergic projections are important for cognitive function (38).

Furthermore, NGF at the target cholinergic neurons affects their survival, fiber growth and expression of transmitter-specific enzymes and able to prevent the degradation of cholinergic neurons in adult rats with experimental lesions mimicking the cholinergic deficit in Alzheimer’s disease (39). These findings suggest that; increasing the availability of NGF to human cholinergic cells might promote their survival in certain disease processes. Also, NGF has a rapid neurotransmitter-like action to regulate cholinergic neurotransmission and neuronal excitability (37). These beneficial effects of NGF on the central cholinergic neurons and on spatial recent memory and cognitive functions can persist for up to one month after discontinuation of treatment (41). Hence, the improvement in both the cognitive tasks and locomotor activities of animals impaired by scopolamine after administration of nerve growth factor loaded nanoparticles indicate the success of nerve growth factor to reach its targets in the CNS, hence produces its above
mentioned beneficial effects.

These findings indicate the ability of PBC-nanoparticles coated with polysorbate-80 to act as a carrier system for neurotrophic peptides transport within CNS particularly for the transport of nerve growth factor. The mechanism of enhancement of drugs and peptide transport into the brain mediated by the PBC-nanoparticles, however, is not fully elucidated. At concentrations of PBC-nanoparticles and polysorbate-80 that achieve significant drug delivery to the brain, there is little in vitro or in vivo evidence to suggest that a generalized toxic effect on the BBB is the primary mechanism for drug delivery to the brain. Nanoparticles-mediated drug transport across BBB depends on the over coating of the nanoparticles with polysorbate derivative, especially polysorbate-80. Studies indicated that, polysorbate-80 coated nanoparticles are taken up by brain endothelial cells much more rapidly and in significantly higher amounts (20-fold) than uncoated nanoparticles. Over coating with these materials seems to lead to the adsorption of apolipoprotein-E from blood plasma onto the nanoparticle surface. Preferential adsorption of ApoE was reported as one important factor for targeting of polysorbate-80 modified poly(butylcyanoacrylate) nanoparticles to the brain. ApoE has been reported as a key factor for the BBB passage where, besides the liver as a major site of ApoE receptors, there are also ApoE receptors present at the BBB. The ability of BBB passage by a sufficient amount of drug loaded nanoparticles is not only due to adsorption of ApoE. Preferential adsorption of ApoA-I and A-IV also play a role. It is probably that, the “team-work” of apolipoproteins like A-I and A-IV prevent the nanoparticles from hepatic uptake prior to their contact with the ApoE receptors at the BBB. The nanoparticles then seem to mimic low density lipoprotein (LDL) particles and could interact with the LDL receptors leading to their uptake by the brain capillary endothelial cells via receptor-mediated endocytosis. After this the drug may be released in these cells and diffuse into the brain interior or the particles may be transcytosed. Other processes such as light junction modulation or P-glycoprotein (Pgp) inhibition also may occur. Moreover, these mechanisms may run in parallel or may be cooperative thus enabling a drug delivery to the brain.

CONCLUSION

- Poly(butylcyanoacrylate) nanoparticles when coated with polysorbate-80 represent a useful carrier and helpful tool for transporting of neurotrophic peptides as NGF through BBB.
- This study represents a framework for further experimental and clinical studies needed to indicate the usefulness of this carrier system for NGF transport for clinical use in human being.

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References


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