NANOTECHNOLOGIES

Antiparkinsonian Effect of Nerve Growth Factor Adsorbed on Polybutylcyanoacrylate Nanoparticles Coated with Polysorbate-80

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The study examined the antiparkinsonian effect of nerve growth factor adsorbed on the surface of polybutylcyanoacrylate nanoparticles coated with polysorbate-80 surfactant. The parkinsonian syndrome in C57B1/6 mice was provoked by intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The basic symptoms of the parkinsonian syndrome decreased under the action of the nerve growth factor adsorbed on nanoparticles coated with polysorbate-80, which was seen from decreased rigidity and increased locomotor activity compared to control mice receiving with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine alone. This effect of nerve growth factor on nanoparticles persisted after 7 and 21 days after single injection of the neurotoxin. These data attest to the possibility of using nanoparticles prepared from amphiphilic polymers and coated with polysorbate-80 for the delivery of nerve growth factor into the brain during systemic treatment.

Key Words: *nerve growth factor; polybutylcyanoacrylate nanoparticles coated with polysorbate-80; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Parkinsonian disease*

A promising avenue in the development of drugs for the treatment of neurodegenerative diseases is the use of neurotrophic factors and specifically, nerve growth factor (NGF), the best studied member of the family of peptide regulators normalizing growth, maturation, and function of neurons in the central and peripheral nervous systems. NGF provides neuroprotection and regeneration of nerve cells [10]; it regulates the development of cytoskeleton, formation and plasticity of synapses, and exocytosis processes [4]. In the brain, NGF is produced mostly by pyramidal neurons of the cortex and by hippocampal and striatal cells [6]. Preclinical and clinical studies attest to considerable promise in the development of the pharmacological tools affecting the neurotrophin system for the treatment of diseases caused by neurodegenerative processes (Alzheimer, Parkinson, and Huntington diseases as well as diabetic neuropathy and other diseases) [3,13]. In parkinsonian patients, NGF level in the blood and substantia nigra decreases in comparison with its level in the frontal cortex, cerebel-

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lum, and other cerebral subdivisions. A protective action of NGF administered into the substantia nigra was demonstrated in rats with parkinsonian syndrome provoked by 6-hydroxydopamine [5].

However, delivery of NGF into CNS is difficult, because this large protein molecule cannot cross the blood-brain barrier [6]. Therefore, the search for a carrier providing targeted delivery of NGF into CNS is of particular importance. One of the most promising carriers are the nanoparticles, colloidal systems composed of polymer matrix incorporating medical preparation and coated with polysorbate-80 (PS-80) preventing their scavenging by cells of the reticuloendothelial system, which prolongs circulation of these particles in the blood and increases their concentration in cerebral vessels (up to 20 fold) [8,12].

In the study of antiamnestic effect of NGF [1] and the effects of loperamide, tubocurarine, and doxorubicin [9], high indices of targeted delivery were obtained with the use of polybutylcyanoacrylate (PBCA) nanoparticles.

Our aim was to study the antiparkinsonian effect of NGF adsorbed on the surface of PBCA nanoparticles (NGF-NP) coated with PS-80 in C57B1/6 mice with modeled Parkinson disease.

MATERIALS AND METHODS

Experiments were carried out on 3-month old male C57B1/6 mice (n=40) weighing 22-24 g. The animals were obtained from Stolbovaya Central Animal Breeding Department (Russian Academy of Sciences) and maintained at 20-22°C in stainless steel cages (10 mice per case) with a 12-h day-night cycle and food and water *ad libitum*. All experiments were conducted in the first half of the day.

The parkinsonian syndrome was provoked by intraperitoneal injection of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 30 mg/kg). This model of MPTP-induced parkinsonian syndrome is the most adequate, because this toxin induces behavioral and biochemical manifestations similar to those observed in humans with Parkinson disease [2,13]. The basic extrapyramidal disturbances were assessed as described elsewhere [2] immediately after MPTP injection and then 90 min, 24 h, 7 day, and 21 day after combined injection of neurotoxin and NGF-NP coated with PS-80.

Oligokinesia was evaluated by changes in motor activity in the open field test (horizontal and vertical movements over 2 min) in an actometer (Ugo Basil) for 10 min. Rigidity was assessed by the gait alterations. To this end, we measured the distance between the footprints left by the front and hind paws in a special box (the step length). Catalepsy was assessed by ability of the mice to stay in a "frozen" state for 45 sec in an uncomfortable posture on the steps.

The study used pharmaceutical physiological saline (0.9% NaCl, Moskhimfarm); MPTP (power, Research Biochemical Inc.); NGF isolated from mouse submaxillary glands (lyophilized powde), 7S Nerve Growth Factor, Mouse Submaxillary Glands, Calbiochem, An Affiliate of Merk KGaA, Darmstadt); PCBA nanoparticles (PCBA-NP, lyophilized powder, Institute of Molecular Medicine, I. M. Sechenov Moscow Medical Academy) and PS-80 (LabTeKh).

Suspension of the nanoparticles with adsorbed NGF and coated with PS-80 was prepared as follows: lyophilized PCBA-NP powder (90 mg) was dispersed in 0.8 ml isotonic NaCl to obtain a homogenous suspension of milky color. Then lyophilized NGF powder (45 μg) was dissolved in 1 ml isotonic NaCl. This solution was mixed with suspension of nanoparticles and incubated at 5-8°C for 3 h. The obtained suspension was supplemented with 1.8 μl 1% PS-80 solution and incubated for 30 min. As a result, 2 ml suspension of 2% PCBA-NP coated with PS-80 and containing 45 μg NGF (5 μg in 0.2 ml) was prepared for injections [1]. NGF-NP coated with PS-80 were prepared immediately before use and were used within one day.

Passive control (intact mice) received physiological saline. Active control mice received a single injection of MTPT (30 mg/kg), and mice of two experimental groups received single intraperitoneal injection of NGF-NP coated with PS-80 (5 μg NGF in 0.2 ml 2% PBCA-NP suspension) either 15 min before MPTP or 10 min after administration of the toxin.

The data were processed with Biostat Windows-compatible software and parametric Student t test.

RESULTS

Dynamic evaluation of locomotor and vegetative functions showed that 2-3 min after injection of MPTP, active control mice demonstrated generalized low- and medium-amplitude tremor lasting for 20-40 min. Piloerection, retro-, and lateropulsion appeared after 3-4.5 min, and after 10-15 min rigidity developed, which manifested in gaitdisturbances, strain of the front and hind limbs, appearance of a "hump", and a decrease in the step length. Oligokinesia progressed and attained maximum after 1.5 h. There were periods of freezing, motor stereotypy, unstable gait, and rotational motions. Rigidity

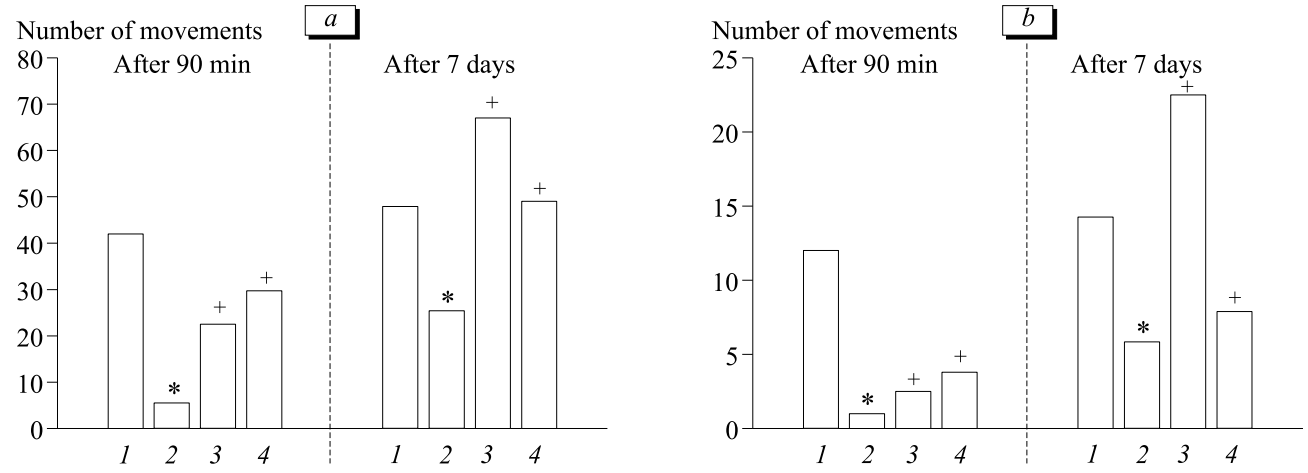

Fig. 1. Effect of NGF-NP coated with PS-80 on MPTP-induced oligokinesia in C57Bl/6 mice (2-min open field test). *a*) horizontal movements; *b*) vertical movements. Here and in Figs. 2 and 3: *1*) physiological saline; *2*) MPTP; *3*) NGF-NP coated with PS-80 before MPTP, *4*) NGF-NP coated with PS-80 after MPTP. $p<0.05$ compared to *intact animals, +active control mice (treated with MPTP only).

and oligokinesia decreased, but persisted after 7 and in 21 days (Figs. 1-3).

Pretreatment of the mice with NGF-NP coated with PS-80 15 min before MPTP considerably decreased some basic parkinsonian symptoms as early as on day 1 of observation. For example, the number of animals with latero- and retropulsion decreased by 37% and that with catalepsy decreased by 34% compared to active control group.

Injection of NGF-NP coated with PS-80 against the background of modeled parkinsonian syndrome also significantly alleviated extrapyramidal disturbances. On day 1, the number of mice with catalepsy and lateropulsion in this group decrased by 83.33 and 39.5%, respectively, compared to active control group. The number of freezing episodes also decreased.

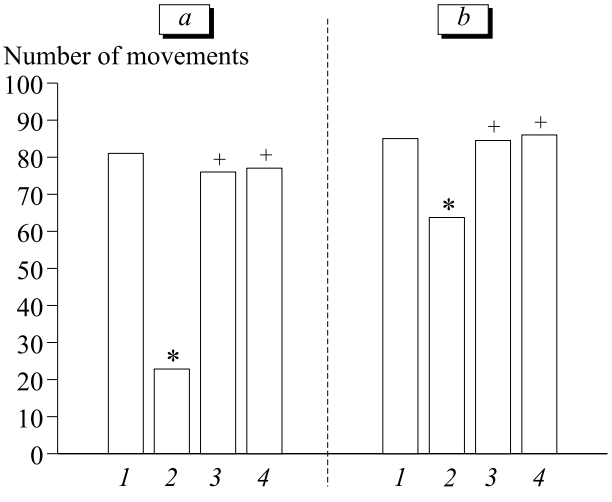

Fig. 2. Effect of NGF-NP coated with PS-80 on MPTP-induced oligokinesia in C57B1/6 mice tested in actometer for 10 min. Here and in Fig. 3: *a*) after 24 h; *b*) after 21 days;

The open field test 90 min after MPTP injection showed that administration of NGF-NP coated with PS-80 (both before and after neurotoxin) moderated symptoms of oligokinesia and significantly increased horizontal and vertical activity (Fig. 1). After 24 h, NGF-NP coated with PS-80 completely eliminated oligokinesia in both experimental groups as assessed by the number of movements in the actometer (Fig. 2). Rigidity also disappeared 24 h after injection of MPTP against the background of NGF-NP coated with PS-80: step length increased by more than 1.6-fold compared to active control group (Fig. 3).

NGF-NP coated with PS-80 produced the antiparkinsonian effect also on the delayed MPTP-induced symptoms observed on postintoxication days 7 and 21. On day 7, this drug restored motor activity in the open field in both experimental groups. It is noteworthy that the total index of vertical and horizontal motor activity in the group receiving NGF-NP coated with PS-80 after MPTP was 1.78-fold higher than in active control group. In the group receiving NGF-NP coated with PS-80 before MPTP this parameter was higher by 2.86 times compared to active control group and even surpassed the corresponding parameter in passive control group (intact animals, Fig. 1).

Measuring motor activity in the actometer on day 21 after MPTP injection showed that NGF-NP coated with PS-80 completely restored motor activity in both experimental groups to the level observed in intact mice (Fig. 2).

On day 21 after administration of MPTP, in animals receiving NGF-NP coated with PS-80 after MPTP the step length increased by 1.12 times compared to active control mice, while in mice recei-

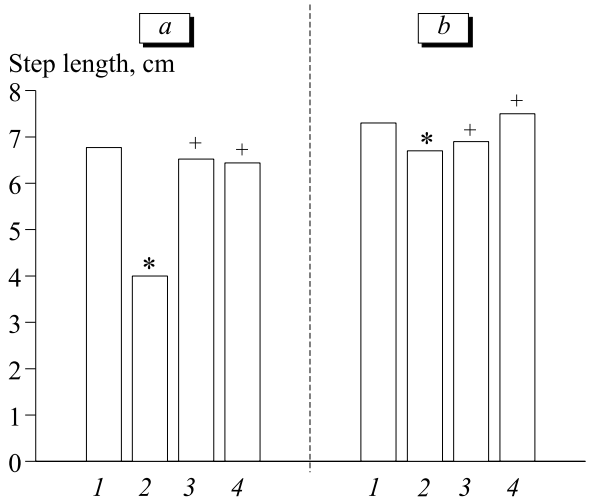

Fig. 3. Effect of NGF-NP coated with PS-80 on MPTP-induced rigidity in C57Bl/6 mice assessed by step length.

ving NGF-NP coated with PS-80 this parameter was close to that in intact animals (Fig. 3).

Our data indicate that intraperitoneal injection of NGF adsorbed on the surface of PBCA-NP coated with PS-80 moderates manifestations of MPTP-provoked parkinsonian syndrome on days 1, 7, and 21 of the experiment.

In recent years, compelling evidence appeared indicating involvement of neurotrophic factors in modulation of dopaminergic transmission in the brain and the potency of NGF to prevent the death of dopaminergic neurons. It was also shown that NGF potentiates dopamine release *in vitro* in a dose-dependent manner. Pronounced elevation in the content of dopamine and homovanillic acid was also observed in the striatum of mice with MPTP-induced parkinsonian syndrome treated with intracerebral NGF [7,11].

Thus, it can be hypothesized that the antiparkinsonian effect of NGF revealed in this study results from modulating action of this factor on the cerebral dopaminergic system. This study provided new data in favor of possibility of using polymer nanoparticles as a vehicle for NGF to cross the blood-brain barrier. These data attest to ability of PBCA-NP coated with PS-80 to carry systemically injected NGF into CNS.

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