PERSPECTIVE

Bacterial melanin in rat models of Parkinson's disease: a potential neuroprotective strategy

Melanins are widely used in medicine, pharmacology and cosmetics. Different technologies have been used to obtain melanin including: chemical synthesis based on oxidation of tyrosine and its derivatives; extraction from animal materials; alkaline extraction from plant material; and microbiological synthesis. A few number of works have been published that were focused on purification of water insoluble 3,4-dihydroxy-phenylalanine-melanins (Kukulianskaia et al., 2002). The majority of synthetic and natural melanins are insoluble in water that significantly complicates preparation of pharmacological and cosmetic preparations. Obtaining of low-cost soluble biotechnological melanin can speed up application of melanin in medicine and other fields. For the first time, melanin-synthesizing strain with high level of pigment synthesis – Bacillus thuringiensis was obtained. The ecologically safe technology of biosynthesis, isolation and purification of the bacterial melanin has been elaborated.

Melanin metabolism disorders can be involved in the etiology of such diseases as parkinsonism, senile macular degeneration, and senile deafness. This pigment is also relevant to the well-known association between pigmentary abnormalities and deafness (Warrensburg's and Usher's syndromes). The Alzheimer's disease and Down syndrome were observed to be also accompanied with pathological disorders in melanin metabolism.

Parkinson's disease (PD) is caused by a deficiency of the neurotransmitter dopamine at the nerve terminals of the nigrostriatal dopaminergic neurons in the striatum, due to selective loss of the dopaminergic neurons in the substantia nigra pars compacta (SNc). Majority of PD cases are sporadic and age related, and approximately 5% are classified as familial disease. Although subject to intensive research, the etiology of PD is not completely studied and the treatment is basically symptomatic. Many factors are speculated to operate in the mechanism of cell death of the nigrostriatal dopaminergic neurons in PD, including oxidative stress and cytotoxicity of reactive oxygen spices, disturbances of intracellular calcium homeostasis, exogenous and endogenous toxins, and mitochondrial dysfunction. Neurodegenerative processes are generally characterized by a long-lasting course of neuronal death.

Evidence of apoptotic cell death in various neuronal and non-neuronal cells was seen through DNA fragmentation and typical morphological changes in PD brains. Although the concept of programmed cell death (apoptosis) in PD is still controversial, data from postmortem brains of PD patients, animal models, and *in vitro* culture studies indicate the presence of apoptotic cell death as well as a proapoptotic environment in the nigrostriatal region in PD (Lev et al., 2003).

Different concentrations of bacterial melanin have been tested in our experiments. It was revealed that the recovery period of motor functions in rats, dosed with various concentrations of bacterial melanin, was different in rats injected with bacterial melanin after unilateral ablation of sensorimotor cortex. In general the recovery of initially elaborated balancing motor reflex (Gevorkyan et al., 2007) occurred earlier in rats injected with low concentrations of bacterial melanin. When injected with low concentrations of melanin (6, 4.5 mg/mL) the recovery periods for instrumental conditioned reflex and balancing limb movements were significantly shorter.

Studies have also shown that neuromelanin containing dopaminergic neurons of the SNc are more subjected to degeneration in patients with PD than dopaminergic neurons that do not contain melanin. The authors have shown that the free extracellular neuromelanin and microgliosis are the main causes of PD. The latest data show that the human extracellular neuromelanin in the absence of microglia itself is not toxic for neurons. But release of neuromelanin from destructed neurons causes the activation of microglia and subsequent neurodegeneration, proving that melanin containing neurons of substantia nigra (SN) are targeted in PD. Bacterial melanin has proved to be non toxic, and it does not cause activation of microglia when applied directly to brain tissue or after injections. It is proved that inflammatory factors may lead to the death of DA neurons in SNc. Bacterial melanin supports the survival of neurons in SNc after induced destruction and preserves dopaminergic cell bodies. Bacterial melanin causes dilation of capillaries in the lesion area, which increases the blood flow in the brain tissue (Petrosyan et al., 2012). Numbers of studies have shown neuroprotective action of melanocyte-stimulating-hormone on motor recovery following central nervous system lesion (Chen et al., 2008).

There are several animal models of PD, such as PD mice produced by repeated injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; hemiparkinsonian rats produced by injecting 6-hydroxydopamine into one side of the ventrotegmental bundle; and electrolytic destruction of dopaminergic neurons in SN. In all experimental series of our project (destruction of cerebellar nuclei, SNc, *etc.*) we have used electrolytic destruction. The same method was also used for the present study. Excitotoxic lesions potentially have advantages over the applied electrolytic destruction, except for the possible chemical interaction and neuropeptide changes.

Potential neuroprotective action of bacterial melanin was studied in rats after unilateral destruction of SNc dopaminergic neurons. Rats were initially trained to an instrumental conditioned reflex and then were subjected to unilateral electrolytic destruction of SNc. Unilateral deficit in balancing hindlimb movements was observed in all rats after the destruction. On the next day after the destruction, part of the animals (experimental group) was intramuscularly injected with bacterial melanin solution at the concentration 6 mg/mL (0.17 g/kg). The other group of operated rats served as a control. On the second day after the operation the testing of instrumental conditioned reflex was resumed in both groups. Comparison of recovery

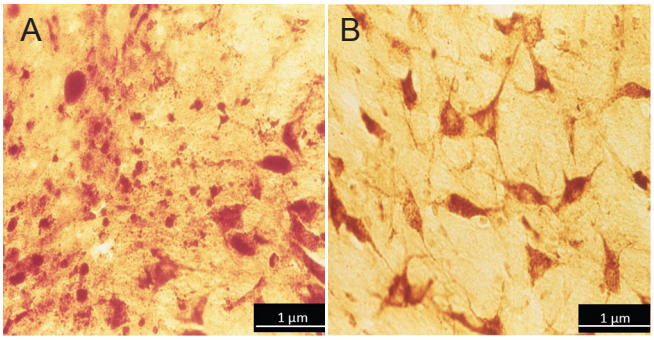

Figure 1 Neurons of substantia nigra pars compacta after destruction.
(A) Midbrain section of control group rat with destructed neurons. (B) Midbrain section of experimental group rat treated with melanin. Survival rate of neurons is high in the section.

periods for the instrumental conditioned reflex in both groups showed that recovery of the reflex and balancing hindlimb movements in melanin treated rats took place in three postoperative testing days, whereas in control group the recovery was not complete after 23 testing days (Petrosyan et al., 2014).

We conducted an electrophysiological study to show the effects of bacterial melanin on the activity of SNc neurons. The spiking rate of neurons was significantly increased by the bacterial melanin injection. Increased electrical activity of SN neurons and prevalence of excitatory responses in dopminergic neurons together with regenerative efforts induced by bacterial melanin accelerate motor recovery after unilateral SNc destruction.

Morpho-histochemical study of brain sections was conducted after the completion of behavioral experiments. It revealed that bacterial melanin stimulates regeneration and microcirculation in SNc in the melanin injected rats (**Figure 1**).

Accelerated motor and behavioral recovery showed that in rats treated with bacterial melanin solution, deficiency of this substance is compensated, and presumably the balance of the substance is restored in the brain, providing fast recovery of locomotion and the initially elaborated instrumental conditioned reflex. The recovery of balancing instrumental conditioned reflex after the destruction of SNc was completed in a very short period of time in rats injected with bacterial melanin solution, suggesting not only the neuroprotective action of bacterial melanin, but also possible compensating role of bacterial melanin in the process of motor control and recovery. The preserved dopaminergic cell bodies and modulating role of melanin contribute to the increased electrical activity of SNc neurons. Electrical activity of SNc neurons is critical for associative learning. The regulatory mechanisms of SNc activity are not completely studied. Such a high frequency activity is not induced or regulated by somatic input.

Bacterial melanin could be a potential biologic medical product for the treatment of PD. Biological compensatory action of bacterial melanin (positive allosteric modulator) and its immunomodulatory effects can ameliorate manifestations of the neurodegenerative disorder. A pharmacokinetic study with isotope labeling has confirmed the ability of bacterial melanin to cross the blood-brain-barrier. The study with radiolabeled melanin confirmed that bacterial melanin is eliminated through liver and kidneys and has a favorable pharmacokinetic profile for use as a therapeutic and neuroprotective agent (Petrosyan and Hovsepyan, 2014a).

Bacterial melanin has been shown to improve significantly the cognitive functions in an animal model of induced acute hypoxia of brain (Petrosyan and Hovsepyan, 2014b). The goal of this study was to test effects of bacterial melanin on memory and motor learning after induced bilateral hypoperfusion. Bacterial melanin significantly affects the water maze test results in rats after bilateral permanent ligation of the common carotid artery. Rats treated with melanin reached the preoperative level of task completion in a very short period compared to control rats.

The potential therapeutic agent for PD is in the stage of preclinical study. Bacterial melanin has been tested as a potent stimulator of regeneration and motor recovery. It stimulates the sprouting of damaged motor tract and peripheral nerve fibers, suppresses secondary inflammation in damaged brain tissue, which is a precondition to use bacterial melanin in graft transplantation. Bacterial melanin dilates vessels and stimulates vascularization. Low concentrations of bacterial melanin (4.5–6 mg/mL) have been shown to be non toxic and not to produce side effects.

Bacterial melanin supports the viability of dopaminergic cell culture. The *in vitro* studies have shown the survival-promoting activity of bacterial melanin on midbrain dopaminergic neurons culture. Bacterial melanin's protective action was studied in MPP^+- and rotenone-induced toxicity cellular model.

The study has also entered the lead optimization phase. To make bacterial melanin more effective and safer we have initiated a study to test effects of bacterial melanin composites with chitosan and its derivatives on the process of motor recovery after unilateral destruction of SNc in rats, with a goal to generate analogues of the initial substance with improved potency, reduced off-target activities, and desirable metabolic properties. The completion of preclinical animal studies and toxicokinetiks will provide data to plan clinical studies.

The compensatory treatment with bacterial melanin in PD will support viability of neurons in nigrostriatal system, stimulate regeneration and restore the level of melanin in cells.

Proposed therapeutic is intended to alter the course of disease progression, prevent aggravation of symptoms and promote healing. The therapeutic strategy will address motor and cognitive symptoms of PD. Effects of bacterial melanin on other non-motor symptoms (speech, mental disturbances) can be evaluated in clinical study.

Tigran Petrosyan*

Department of Kinesiology, Armenian State Institute of Physical Education, Yerevan, Armenia

***Correspondence to:** *Tigran Petrosyan, Ph.D., tigpetrosyan@mail.ru*
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