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Bacterial Melanin as a Potential Targeted Therapy for the Parkinson's Disease

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Target Rationale

Bacterial melanin has a neuroprotective action and the compensatory treatment with BM in models of PD supports viability of neurons in nigrostriatal system, stimulates regeneration and restores the level of melanin in cells.

Bacterial melanin could be a potential biologic medical product for the treatment of Parkinson's disease. Biological *compensatory action* of BM (positive allosteric modulator) and its immunomodulatory effects can ameliorate manifestations of the neurodegenerative disorder.

At Armenian Institute of Biotechnology we have obtained a melanin-synthesizing strain of *Bacillus thuringiensis* - with high level of pigment synthesis. The ecologically safe technology of biosynthesis, isolation and purification of the bacterial melanin (BM) has been elaborated in the Institute [1]. When melanin is produced in preparative quantities its cost, according to the preliminary calculations, is considerably lower than that of the synthetic melanin or of the pigment isolated from other sources. Biotechnologically obtained purified bacterial melanin exhibited a similar infrared absorption spectrum to synthetic melanin and contained quinolic and phenolic structures and an amino acid content of around 20% after acid hydrolysis [1].

Bacterial melanin has been tested in a number of animal models of neurodegeneration, including models of Parkinson's disease [2]. It accelerates motor recovery after CNS lesion, stimulates regeneration in damaged area of brain, has an anti-inflammatory action, dilates capillaries and increases vascularization. In a model of PD with substantia nigra destruction BM accelerated behavioral and motor recovery in rats [3]. It increases electrical activity of dopaminergic neurons in Substantia Nigra pars compacta, which in turn facilitates motor recovery [4]. BM supports motor recovery in the experimental model of encephalomyelitis, exhibits immunomodulatory action [5]. A pharmacokinetic study with isotope labeling has confirmed the ability of BM to cross the blood-brain-barrier (BBB). The study with radiolabeled melanin confirmed that BM is *eliminated through liver and kidneys* and has a favorable pharmacokinetic profile for use as a therapeutic and neuroprotective agent [6]. This peculiarity of BM, to cross the BBB, strengthens the potential protective and anti-apoptotic action of the substance.

In the experiments using laboratory animals with brain surgical trauma it was revealed that BM facilitated recovery of instrumental (operant) reflexes after unilateral ablation of sensorimotor cortex that had caused paresis of limbs [7]. BM accelerated the recovery of physiological functions after nervous tissue damage. Results of brain morpho-histochemical studies revealed a series of factors facilitating the regeneration: intensive vascularization, glia proliferation, decrease in macrophage number, suppression of connective scarring and restraint of inflammatory processes (Figure 1).

In the experiments on laboratory animals (Wistar rats) with brain surgical trauma (destruction of substantia nigra, destruction of sensorimotor cortex, destruction of lateral cerebellar nuclei, damaged corticospinal tract and damaged cortico-rubrospinal tract) [2,7,8-11] it was revealed that BM facilitated the recovery of instrumental conditioned reflexes after that had caused paresis of limbs. Low concentrations of BM accelerated the recovery of physiological functions lost because of nervous tissue damage (Table 1) and were applied in all series of experiments.

BM stimulates axonal sprouting and regeneration. It stimulates the regeneration of damaged peripheral nerve and motor tract [10,12]. BM can be used in graft transplantation as a supporting treatment.

The potential therapeutic agent is in the stage of preclinical study. The next development stage includes *in vitro* studies on dopaminergic neuronal cell culture and endothelial cell culture to clarify effects observed in animal (*in vivo*) models. *In vitro* studies will evaluate the anti- apoptotic and protective role of BM, its effects on endothelial cell culture. The studies are a good tool to evaluate the toxicity of the substance.

Proposed therapeutic is intended to alter the course of disease progression, prevent aggravation of symptoms and promote healing. It address motor and cognitive symptoms of PD, as bacterial melanin has been shown to improve significantly cognitive functions in an animal model of induced acute hypoxia of brain [13]. Effects of BM on other non-motor symptoms (speech, mental disturbances) can be evaluated in clinical studies.

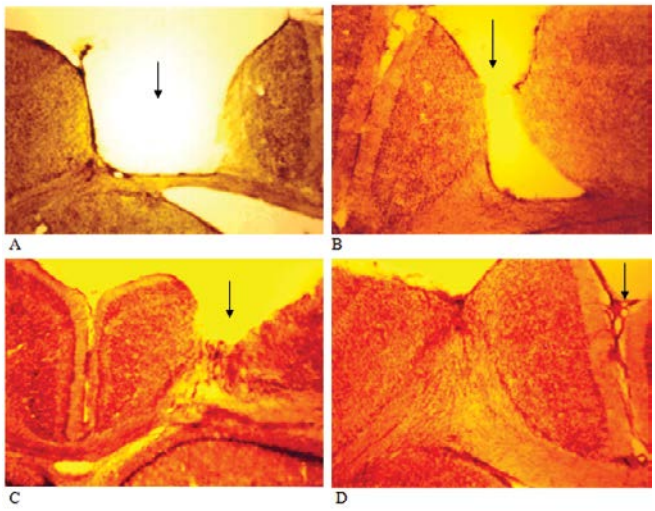

Figure 1: Ablation area of sensorimotor cortex in control rats (A - indicated by the arrow). Ablation area of sensorimotor cortex in experimental rats treated with bacterial melanin (6 mg/ml, 170 mg/kg (B, C, D - indicated by the arrow). Brain sections were obtained one month after the surgery. Meliksetyan's method was used to identify acid phosphatase activity in brain tissue [7,8]. Magnification: ocular: 10, objective:2,5.

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Group of animals		Time to acquisition of the OCR, experimental days	Time to recovery of the OCR after ablation of the synaptic, experimental days	Time to recovery of hindlimb movements after ablation of the sensorimotor cortex, experimental days
A	Control group (n = 6)	2.1 ± 0.75	16 ± 2.2	Incomplete recovery
B	Experimental, given bacterial melanin at a dose of 9,4 mg/ml (n = 6)	3.5 ± 2.1	12.8 ± 4.8	19 ± 4.4
C	Experimental, given bacterial melanin at a dose of 6 mg/ml (n = 6)	2.8 ± 1.3	6.8 ± 1.03	10.2 ± 2.3
D	Experimental, given bacterial melanin at a dose of 5,4 mg/ml (n = 6)	2.1 ± 1.1	9.2 ± 4.8	19.6 ± 7.6

Table 1: Mean Data for Times of Acquisition of the Operant Conditioned Reflex and Its Recovery in Rats Subjected to Unilateral Ablation of the Sensorimotor Cortex (A) and in Rats Treated with Different Concentrations of BT-Melanin Solution (groups B, C, D) [7].

Pharmacokinetic Profile of Bacterial Melanin

Basic pharmacokinetic data have been collected for the bacterial melanin. BM and its metabolites cross the blood–brain barrier [6]. BM shows higher Cmax after intramuscular (i/m; 6 mg/ml) injection, while a long retention was registered after intraperitoneal (i/p; 6 mg/ml) injection. BM is enzymatically stable in blood and in brain parenchyma and is transported by a saturable mechanism into the CNS parenchyma. Uptake from blood occurs throughout the CNS and is particularly high for the substantia nigra, hypothalamus, thalamus and lumbar spinal cord. Radioactively labeled BM is more stable in brain, suggesting that BM introduced into the blood or peripheral tissue (intramuscular injection) could contribute to the levels of melanin in CNS parenchyma. There is not much data on the transport of melanin and its mediators. However, Berliner et al. mentioned the possible role of melanin as a transporter that crosses the BBB and has a potential for pharmacological application as a transporter [14]. To add more information to the pharmacokinetic profile of the BM we also tested the uptake rate of I-BM into liver and kidneys. Results showed that uptake rate was almost two-fold higher in kidneys, meaning that bacterial melanin accumulates in the kidneys and less so in the liver.

Next Step in the Preclinical Study

The next stage of our research project includes *in vitro* studies on dopaminergic neuronal cell culture to clarify effects observed in animal (*in vivo*) models. *In vitro* studies will evaluate the protective role of BM. Based on the previous studies with BM we have hypothesized that BM does not activate microglia. Our research is aimed to test whether TGFb1 is able to inhibit melanin-mediated activation of microglia. The studies are a good tool to evaluate the toxicity of the substance. Testing of induced neuronal spiking activity in neuronal culture treated with different channel blockers will help to identify the mechanism of activating influence of BM on dopaniergic neurons.

The project has also entered the lead optimisation phase. To make BM more effective and safer we have initiated a study to test effects of BM composites with chitosan and its derivatives on the process of motor recovery after unilateral destruction of Substantia Nigra pars compacta in rats, with a goal to generate analogues of the initial substance with improved potency, reduced off-target activities, and desirable metabolic properties. The next development stage includes *in vitro* studies on dopaminergic neuronal cell culture and endothelial cell culture to clarify effects observed in animal (*in vivo*) models. *In vitro* studies will evaluate the anti-apoptotic and protective role of BM, its effects on endothelial cell culture. The studies are a good tool to evaluate the toxicity of the substance. PK/PD studies in animals are part of the project to prospectively predict the human efficacious doses. The completion of preclinical animal studies and toxicokinetics will provide data to plan clinical studies.

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