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PROTECTIVE EFFECT OF BACTERIAL MELANIN ON SUBSTANTIA NIGRA IN PARKINSON'S DISEASE

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Abstract

Parkinson's (PD) disease is a common movement disorder in a wide range of neurodegenerative diseases, often associated with gradual personality degradation. The major reason for PD is the progressive disturbance of dopaminergic neurons of the substantia nigra pars compacta. Therefore, therapy aimed at slowing the death of dopaminergic neurons can be effective. In the treatment of neurodegenerative diseases, various kinds of neuroprotectors are successfully used, after which the recovery of lost functions of the central nervous system is accelerated. In the present study, bacterial melanin was used for this purpose.

A comparative study of the morphofunctional state of the cell structures of the compact substantia nigra in rats was conducted on the rotenone model of PD and in combination with the administration of bacterial melanin.

For the morphological and histochemical study, we used the method of detecting the activity of Ca^{2+} - dependent acid phosphatase.

The data analysis showed that during rotenone intoxication of the brain, neuronal death and substantia nigra depigmentation are observed, sharp morphological changes in intracellular structures occur, which indicates gross metabolic and morphological

disorders. With the introduction of bacterial melanin, there is a tendency to preserve the typical morphological picture of the neurons of the substantia nigra pars compacta compared to the model of PD. Obtained data give reason to suggest bacterial melanin acts as a neuroprotective agent.

Keywords and phrases
Substantia nigra pars compacta, bacterial melanin, PD model.

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Համառոտագիր

Պարկինսոնի հիվանությունը համարվում է լայն տարածում ունեցող շարժողական խանգարում և դասվում է նեյրոդեգեներատիվ հիվանդությունների շարքին: Այն հաճախ կապված է անձի անհատականության աստիճանական խաթարման հետ: Պարկինսոնի հիվանդության հիմնական պատճառներն են սև նյութի կոմպակտ մասի դոֆամիներգիկ նյարդաբջիջների պրոգրեսիվ քայքայումն ու մահը: Դոֆամիներգիկ նյարդաբջիջների մահվան դանդաղեցումը կարող է արդյունավետ բուժման եղանակ լինել: Նեյրոդեգեներատիվ հիվանդությունների բուժման համար կիրառում են տարբեր նյարդապաշտպան միջոցներ, որոնց

ներարկումը արագացնում է կենտրոնական նյարդային համակարգի կառույցների գործառույթների վերականգնումը: Կատարվել է առնետների սև նյութի կոմպակտ մասի բջջային կառույցների մորֆոֆունկցիոնալ վիճակի համեմատական հետազոտություն Պարկինսոնի հիվանդության մոդելում՝ համակցված բակտերիալ մելանինի ներարկմամբ: Մորֆոհիստաքիմիական ուսումնասիրության համար օգտագործվել է Ca^{2+} կախյալ թթու ֆոսֆատազի ակտիվության հայտնաբերման մեթոդը: Ստացված տվյալները ցույց են տվել, որ ուղեղի ռոտենոնային թունավորման ժամանակ նկատվում են նեյրոնի մահ և սև նյութի գունազրկում, տեղի են ունենում ներբջջային կառուցվածքների կտրուկ մորֆոլոգիական փոփոխություններ, ինչը վկայում է նյութափոխանակության և մորֆոլոգիական կոպիտ խանգարումների մասին: Իսկ բակտերիալ մելանինի ներարկման ժամանակ դիտվում է սև նյութի կոմպակտ մասի նյարդաբջիջների նորմալ մորֆոֆունկցիոնալ պատկերի պահպանման միտում՝ ի տարբերություն Պարկինսոնի հիվանդության մոդելի: Ստացված տվյալները հիմք են հանդիսանում եզրակացնելու, որ բակտերիալ մելանինը գործում է որպես նյարդապաշտպան գործոն:

Բանալի բառեր և բառակապակցություններ

Սև նյութի կոմպակտ բաժին, բակտերիալ մելանին, Պարկինսոնի հիվանդության մոդել:

ЗАЩИТНОЕ ДЕЙСТВИЕ БАКТЕРИАЛЬНОГО МЕЛАНИНА НА ЧЕРНУЮ СУБСТАНЦИЮ ПРИ БОЛЕЗНИ ПАРКИНСОНА

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Аннотация

Болезнь Паркинсона является распространенным двигательным расстройством в широком спектре нейродегенеративных заболеваний, нередко связанная с постепенной деградацией личности. Основной причиной болезни Паркинсона является прогрессирующее разрушение и гибель дофаминергических нейронов компактной части чёрной субстанции. Поэтому эффективной может стать терапия, направленная на замедление гибели дофаминергических нейронов. При лечении нейродегенеративных заболеваний успешно применяются разного рода нейропротекторы, после введения которых ускоряется восстановление утраченных функций структур центральной нервной системы. В настоящем исследовании с этой целью был использован бактериальный меланин. Было проведено сравнительное изучение морфофункционального состояния клеточных структур компактной части чёрной субстанции крыс на ротеноновой модели болезни Паркинсона и в сочетании с введением бактериального меланина. Для морфогистохимического исследования использовали метод выявления активности Ca^{2+}- зависимой кислой фосфатазы. Анализ полученных данных показал, что при ротеноновой интоксикации мозга отмечаются гибель нейронов и депигментация черной субстанции, происходят резкие морфологические изменения внутриклеточных структур, что свидетельствует о грубых метаболических и морфологических нарушениях. При введении же бактериального меланина наблюдается тенденция к сохранению нормальной морфологической картины нейронов компактной части чёрной субстанции по сравнению с моделью болезни Паркинсона. Полученные данные дают основание полагать, что бактериальный меланин действуют в качестве нейропротекторного агента.

Ключевые слова и фразы

Компактная часть черной субстанции, бактериальный меланин, модель болезни Паркинсона.

Introduction

Parkinson' disease is the most common form of parkinsonism, a group of neurological disorders associated with movement problems such as stiffness, sluggishness, and tremors. More than 6 million people worldwide suffer from PD. It is a heterogeneous disease with rapidly and slowly progressive forms [1]. Studies have shown that the main cause of PD is the progressive destruction and death of neurons that produce the neurotransmitter dopamine [2], which are located in the substantia nigra pars compacta (SNc), as well as their nerve endings located in the striatum [3]. Damage to nerve cells occurs according to the so-called abiotrophic type, manifested in a progressive loss of viability, gradual degeneration, leading to pathological conditions and loss of functions, and are mainly manifested in the formations included in the extrapyramidal and vegetative nervous systems [4].

Dopaminergic neurons SNc are pigmented due to the accumulation of oxidative metabolism of the neurotransmitter dopamine, the precursor neuromelanin. Neuromelanin in SNc is sensitive to a wide range of toxic agents, acting as a system capable of providing neuronal damage [5]. It has been established that inflammatory factors can lead to the death of dopaminergic SNc neurons. Acceleration of death of dopaminergic neurons SNc is triggered by the processes that include sources of oxidative stress [6]. Combination therapy aimed at slowing the death of dopaminergic neurons and excluding the progression of non-dopaminergic symptoms that develop in the last stage of PD can be effective [7]. In recent decades, various types of neuroprotectors have been successfully used in the treatment of neurodegenerative diseases, after the introduction of which the restoration of the lost functions of the structures of the central nervous system is accelerated. In the present study, bacterial melanin (BM) was used for this purpose.

The water-soluble BM obtained biotechnologically can be a potential biological, medical product for treating neurodegenerative diseases (including PD). BM has a high biological activity and bio stimulating effect [8]. The example of the lateral hypothalamus of rats showed that the melanin-concentrating hormone suppresses the synaptic activity of glutamate and GABAergic neurons [9]. BM can promote the survival of neurons in SN under the influence of toxic factors and suppress the inflammatory process [10]. The restoration of the balancing movement of the paralyzed limb in rats was studied using various models of neurodegeneration. It was shown that the introduction of BM reduces the recovery time of the conditioned reflex several times. The ability of melanin to stimulate regeneration processes in the nervous tissue was proved, and it was shown that BM promotes sprouting of nerve fibres, restores conductivity in the motor tract after its damage. Under the influence of BM, vascularization of the cortex is enhanced

due to the expansion of the lumen of the vessels and the opening of new branches in the capillary network, which leads to a significant increase in brain tropism, ensures the safety of nerve cells, which contributes to the rapid restoration of impaired motor functions [11]. BM may promote the rapid activation of stem cell division since it exhibits those qualities necessary for the structural and functional recovery of the central nervous system after trauma [12]. Studies using BM have shown that the substance does not cause microgliosis and has no toxic side effects [13].

This work aimed to study the effect of BM on the morphofunctional state of the substantia nigra using an experimental model of PD. A morphofunctional study of the compact part of the substantia nigra of intact rats after rotenone intoxication (PD model) and in combination with BM injections was carried out.

Material and methods

The rotenone PD model is used to study the mechanisms of damage to dopaminergic neurons and assess neurochemical, behavioural, and cognitive manifestations, particularly up to 4 weeks of survival [14]. The experiments were carried out on 15 white male rats (220-250 g.) in three series:

1) intact rats (n = 5); 2) on the model of PD induced by unilateral administration of rotenone and kept for 4 weeks (n = 5); and 3) unilaterally injected with rotenone, similar to series (2), in combination with i.p. injection of bacterial melanin (175 mg / kg, optimal tolerated dose for rats) every other day for 4 weeks - 7 injections (n =5). Rotenone was administered under anesthesia (pentobarbital, 40 mg / kg, i.p., 12 μg in 0.5 μl Dimexide at a rate of 1 μl / min) in a medial forebrain bundle at coordinates (AP + 0.2; L ± 1.8; DV + 8 mm) stereotaxic atlas [15]. The animals were kept under the same conditions during the entire postoperative time (4 weeks) before the acute experiment. The experimental protocol satisfied the provisions of the European Communities Council Directive (2010/63/UE). The Ethics Committee approved Yerevan State Medical University after Mkhitar Heratsi (N4 IRB APPROVAL, 15 November 2018).

Morphological and histochemical studies were carried out by the method of revealing of

Ca^{2+}-dependent acid phosphatase activity [16]. The method is a variation of Nissl staining and Golgi silver impregnation. For morphohistochemical study in animals under anaesthesia (40 mg/kg, i.p.), the brain was taken, which was fixed in a 5% solution of neutral formalin, prepared in 0.1 M phosphate buffer (pH = 7.4), for 48 hours at + 4 ° C. Frozen frontal sections, 40-50 μm thick, were prepared from the brain blocks, which, according to the requirements of further processing, were transferred into a freshly prepared incubation mixture. The finished preparations were analyzed under a

light-optical microscope. The subsequent shooting of the obtained preparations was carried out using a photo attachment (*Model FMA050, Amscope MU800 8 MP (USA*) through a microscope *OPTON* (*West Germany*).

Results and discussion

The substantia nigra refers to the extrapyramidal system. It has a complex structure and abundant blood supply, which indicates the significant role of its components in the coordination system of vital functions. It consists of three parts, the main of which are the pars reticulate and pars compacta (Fig. 1 a). Analysis of morphohistochemical data showed that in intact animals, triangular, elongated and polygonal neurons, rich in neuromelanin pigment, are found in the SNc (Fig. 1 A-C). Fine-grained or clumpy melanin diffusely fills almost the entire cytoplasm of SNc nerve cells (Fig. 1 C). Nerve cells have long axons, several branching processes (Fig. 1 B, C). In the axons of multipolar SNc cells, alternating light and dark areas are observed, which gives the impression of cross-striation (Fig. 1 C). Most likely, these dark areas correspond to regions of high acid phosphatase activity. SNc neurons are intensely coloured, which is explained by a high level of metabolism, which, apparently, is associated with the pigment-forming function of this structure [17]. Among SNc neurons, a moderate reaction of glial cell nuclei is observed (Fig. 1 A), which is characteristic of the norm.

Under conditions of rotenone intoxication, there is a decrease in the density of SNc neurons, a violation of cytoarchitectonics compared to the norm (Fig. 1 D, E). In the cytoplasm of the nerve cell, lysis of the granules of the chromatophilic substance is noted up to its clarification. Neuronal damage occurs in varying degrees of severity (Fig. 1 E, F). There is depigmentation of SNc neurons, and there are cells with a complete absence of melanin. That is, there is a release of the melanin from damaged cells. The cells have a disrupted characteristic polygonal shape; there is no clear delineation of cell groups (Fig. 1 E, F). Some polygonal SNc cells acquire a spherical shape because they swell, the cell body increases in size and processes shorten or disappear (Fig. 1 F). But most neurons have an elongated or triangular shape, the contours of the cells become irregular, indistinct, the border between the body and processes is not visible. They react with long shoots, in which the precipitate of lead phosphate is dusty or fine-grained. The cytoplasm shows a swollen nucleus with an eccentrically located dark nucleolus (Fig. 1 F). In some neurons, a coarse-clumpy precipitate of lead phosphate is unevenly distributed in the cell body, which indicates a possible complete decay; dendrites are thickened and shortened. Among degenerated SNc neurons, homogeneously stained nuclei of glial cells are clearly distinguished in significant numbers (Fig. 1 E, F).

Thus, with rotenone intoxication of the brain, neuronal death and SNc depigmentation are noted, and abrupt morphological changes in intracellular structures occur, indicating gross metabolic and morphological disorders. There are different degrees of damage to the SN cell structures. The morphological picture of SNc neurons after rotenone intoxication reveals changes in the nerve cell's primary stimulation. Usually, this condition is a reversible cellular process; however, with the deepening of the neurodegenerative process, the cell can die, undergoing vacuolization, lysis or pycnosis.

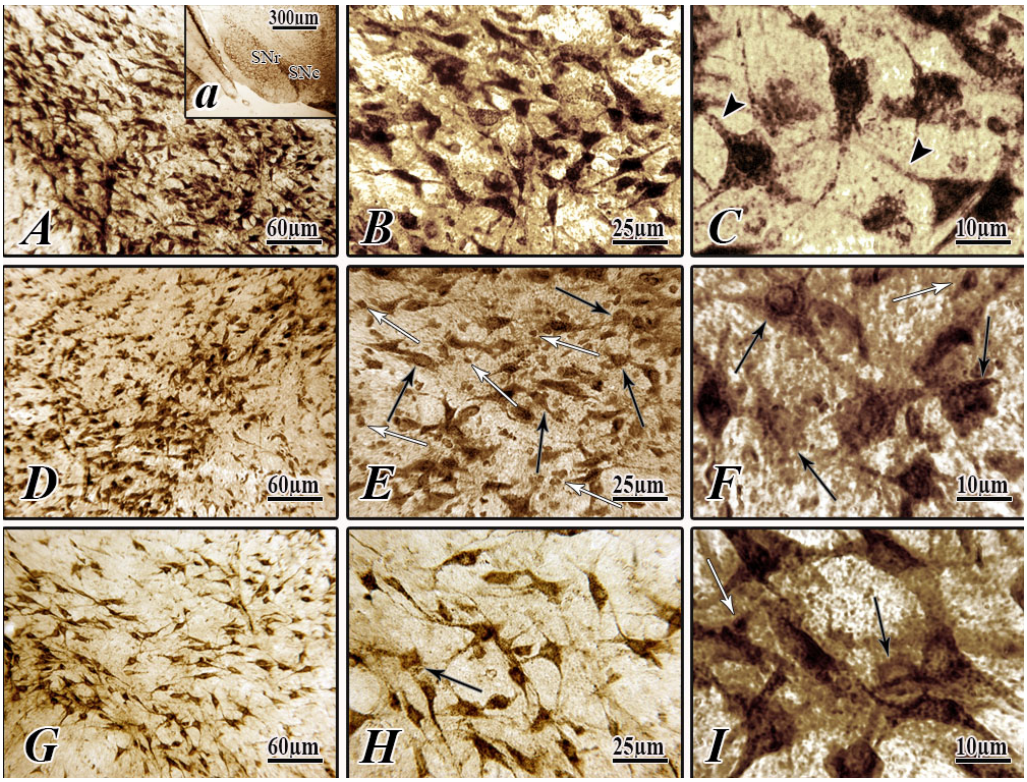

Figure 1. Micrographs of cellular structures of the substantia nigra par compacta of intact (A-C) and experimental (D-I) rats (D-F - in conditions of rotenone intoxication; G-I - in conditions of rotenone intoxication in combination with regular administration of bacterial melanin). D-F - violation of the shape, size and contours of cells, clarification of the cytoplasm, gliosis; G-I – positive changes in the structural properties of neurons, high phosphatase activity in the cytoplasm and processes of neurons, clear contours) (black arrow - chromatolysis; white arrow - glial nuclei; black head of the arrow - cross-striation in the processes of neurons). SNc - the substantia nigra pars compacta; SNr - substantia nigra pars reticulate.

Magnification.: ×25 (a); ×160 (A, D, G); ×400 (B, E, H); ×1000 (C, F, I).

In animals that received an injection of rotenone in combination with melanin injections, the characteristic shapes and sizes of SNc cells were largely preserved. The cytoplasm is intensely coloured; a light-coloured centrally located nucleus is visible in it. Neurons show thin, long processes (Fig. 1 G-I). In SNc, along with cells that retained their shape and size, affected neurons are rarely detected. CP activity is weak, processes are not detected, but the nucleus is centrally located (Fig. 1 I).

In some cases, cells with short processes are observed; they are light -coloured, and their nuclei are eccentrically located (Fig. 1 H). In general, under the influence of bacterial melanin, the morphological picture of SN neurons is observed, which is close to normal. In the intercellular space, against the background of preserved or partially damaged SN neurons, glial nuclei are locally found. A protective reaction of glial cells in relation to neurons occurs (Fig. 1 I).

Conclusions

Research results show that lesions of the neurons of the substantia nigra pars compacta in Parkinson's disease occur according to the so-called abiotrophic type [18]. The affected nerve cells undergo pronounced atrophy, significant changes in the cytoplasm and nucleus occur inside the cells, and pathological changes in the neuroglia are noted. With the regular introduction of bacterial melanin after rotenone injection, positive changes in the structural properties of neurons are observed in the substantia nigra pars compacta compared to the rotenone model of PD. The morphological picture of neurons is close to normal, the shape and size of the cells are preserved, the reaction of the glial nuclei is moderate. In most neurons of the substantia nigra, long processes react, which is important for maintaining intercellular contacts. An increase in phosphatase activity is observed in the cytoplasm of cells, which indicates the activation of metabolic processes aimed at maintaining the body's homeostasis, disturbed as a result of rotenone intoxication. The therapeutic effect of bacterial melanin use is apparently due to a beneficial effect on the modulation of the secondary inflammatory process and inhibition of microgliosis, and an improvement in the tropism of brain tissue [19]. The data obtained allow us to conclude that bacterial melanin plays a certain neuroprotective role, accelerating compensatory recovery in the central nervous system against the background of developing neurodegenerative changes inherent in PD.

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Հոդվածը ներկայացվել է տպագրության 15.03.2021 թ.,
ուղարկվել է գրախոսության 31.03.2021 թ., ընդունվել է տպագրության
02.04.2021 թ.: