Therapeutic Efficacy of the Neuroprotective Plant Adaptogen in Neurodegenerative Disease (Parkinson's Disease as an Example)

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 6, pp. 619-621, June, 2010
Original article submitted April 08, 2010

Therapeutic efficacy of the plant neuroprotector Phytomix-40 in Parkinson's disease was demonstrated. This preparation consists of the components from extracts of 40 plants, including some adaptogens (ginseng, eleutherococcus, *Rhodiola rosea*, *etc*.). The preparation normalized immune, antioxidant, and hormonal parameters in patients. The neuroprotective plant adaptogen can be used in complex therapy for Parkinson's disease for improving its efficacy.

Key Words: *Parkinson's disease; neuroprotectors; adaptogens; immunomodulatory action*

Parkinson's disease (PD) is a chronic disorder associated with the progressive death of dopaminergic neurons in the substance nigra. This process is determined by specific genetic disturbances in brain cells.

The multifactorial pathogenesis of PD necessitates the development of combination pathogenetic therapy (CPT) for this disease [4]. The efficacy of CPT is low due to the absence of neuroprotectors that can prevent degeneration of dopaminergic neurons in the substance nigra [6].

The complex plant adaptogen Phytomix-40 (PM-40) includes components from extracts of *Rhodiola rosea*, eleutherococcus, ginseng, and other adaptogens with neuroprotective properties. Our previous experiments showed that oral administration of PM-40 increases motor activity and reduces the severity of rigidity in mice with MPTP-induced Parkinson's syndrome. Moreover, PM-40 prevented sharp decrease in the content of dopamine and its metabolites (dihydroxyphenylacetic acid [DOPAC] and homovanillic acid) in the striatum of mice with MPTP-induced Parkinson's syndrome. The content of toxic LPO metabolite malonic dialdehyde (MDA) in the striatum and homogenate of mouse brain was shown to decrease to normal after PM-40 treatment [1]. These experiments formed the basis for clinical study of PM-40 and evaluation of the possibility of using this drug for improving the efficacy of CPT for PD.

Here we studied the effects of PM-40 on clinical characterisitcs of motor activity. We also estimated the effect of PM-40 on some protective systems (immune and antioxidant systems; and concentration of the stress hormone cortisol) playing a role in the pathogenesis of PD.

MATERIALS AND METHODS

PM-40 includes components of ginseng, *Rhodiola rosea*, magnolia vine, *Eleutherococcus senticoccus*, strawflower, eucalyptus, dandelion, hawthorn, blackcurrant, *etc*. The composition is protected by the inventor's certificate of Russian Federation [3]. PM-40 is certified as a parapharmaceutical.

We examined 32 patients (43-78 years, average age 60.8±2.0 years) with the diagnosis of PD. The

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0007-4888/10/1496-0682

control group consisted of healthy volunteers of the same age. The average duration of PD was 5.2±0.7 years (minimum duration 1 year, maximum duration 21 years). The shaking-rigid and rigid-shaking forms of this disease were observed in 16 and 15 patients respectively. One patient had the akinetic-rigid form. The reference group included the patients of a similar age (n=41) with no neurological diseases. The patients received a combination antiparkinsonian therapy, which included L-DOPA-containing drugs (Nakom, Sinemet CR, Madopar), PK-Merc, Mirapex, phenazepam, vitamin E, *etc*. The mean group dose of L-DOPA-containing drugs over the period of treatment (5.2±0.7 year) was 411.5±134.6 g per patient (minimum dose 1 g, maximum dose 1370 g). Clinical and laboratory observations were performed for 3 months. Two courses of PM-40 therapy were conducted at a 2-weel interval. The drug in a daily dose of 45 ml/75 kg was given for 1.5 months (15 ml drug and 5 ml water, 15-20 min before eating, 3 times daily). The patients were subjected to clinical and laboratory examination by the end of 2 courses of therapy. The unified Parkinson's disease rating scale (UPDRS) was used for an objective evaluation of neurological disorders. The total score was calculated for sections II (disturbances in daily activity) and III (motor disorders in PD patients). Immune parameters (number of lymphocytes with expression of differentiation antigens CD3, CD4, CD8, CD16, and CD20, activation antigens CD25, HLA-DR, and CD95, and adhesion molecules CD11b and CD18) were measured in a fluorescence immunoassay. The relative content of lymphocytes expressing these antigens was measured on a FACScan flow cytofluorometer (Becton Dickinson) in the lymphocyte gate. The concentration of cytokines IL-6, TNF-α, and IFN-γ was measured by enzyme-linked immunosorbent assay using Tsitokin kits. Serum cortisol concentration in PD patients was measured by an enzyme-linked immunosorbent assay with Roche and Orion Diagnostics kits. The content of MDA, activities of superoxide dismutase (SOD), catalase, and glutathione-S-transferase, and amount of glutathione in the blood were measured by standard biochemical methods.

The results were analyzed by one-way analysis of variance (ANOVA), Newman–Keuls test, and nonparametric Kruskal–Wallis ANOVA followed by Mann–Whitney test.

RESULTS

The total scores of sections II (disturbances in daily activity) and III (motor disorders) in PD patients before PM-40 treatment were 19.2±1.4 and 23.8±1.8, respectively. The severities of tremor, rigidity, and bradykinesia were 2.2±0.3, 2.20±1.14, and 2.00±0.16, respectively.

After combination therapy with PM-40 the total score of disturbances in daily activity was reduced to 14.6±1.1 points. The total score of motor disorders was also decreased to 18.9±1.5. The degree of tremor and rigidity in PD patients decreased by 0.9 and 0.5 points, respectively. The severity of bradykinesia decreased by 0.5 points. The degree of postural tremor and rest tremor was reduced. We revealed a decrease in the amplitude and periodicity of tremor.

During the initial examination, MDA content in the blood from PD patients was 6.8±0.3 μmol/ml (1.6-fold higher compared to normal). Despite treatment with standard antiparkinsonian drugs, MDA content in these patients remained unchanged for 3 months. However, SOD activity in PD patients was lower compared to normal (375.4±20.4 and 420.2±18.5 U/ml/min, respectively). Activities of antioxidant enzymes catalase and glutathione-S-transferase and content of glutathione in PD patients (31.3±1.5 U/ml/min, 1.24±0.10 μmol/liter/min, and 1.39±0.10 μmol/liter, respectively) were lower compared to normal (32.8±0.9 U/ml/min, 1.37±0.06 μmol/liter/min, and 1.69±0.12 μmol/liter, respectively). Treatment with PM-40 was followed by a significant decrease in MDA content in the blood of patients (4.2±0.3 μmol/ml), which approached the mean level of LPO in healthy volunteers. We revealed a significant increase in activities of SOD (463.3±15.6 U/ml/min), catalase (36.1±1.4 U/ml/min), glutathione-S-transferase (1.7±0.1 μmol/liter/min), and glutathione (1.78±0.20 μmol/liter).

The initial examination of immune parameters in PD patients receiving standard antiparkinsonian therapy revealed reduced total number of T lymphocytes ($CD3^+$, 51.5±1.6%), helper/inductor cells ($CD4^+$, 28.0±0.9%), B lymphocytes ($CD20^+$, 4.3±0.5%), and HLA-DR antigen-expressing cells (8.9±1.1%). These changes are typical of immunosuppression. However, other parameters in PD patients were higher compared to the control. It concerns the number of $CD11b^+$ macrophages (22.0±3.0 and 11.9±0.8%, respectively), $CD18^+$ macrophages (84.1±3.7 and 56.8±1.0%, respectively), natural killer cells ($CD16^+$ lymphocytes; 15.9±1.5 and 11.4±0.5%, respectively), cells expressing the IL-2 receptor ligand ($CD25^+$; 6.1±1.1 and 3.1±0.4%, respectively), and cells expressing the apoptotic antigen ($CD95^+$; 35.4±2.3 and 3.1±0.4%, respectively). The elevated parameters are associated with proinflammatory activation of the immune system, which results from inflammation of the microglia during PD [7]. These parameters remained practically unchanged after 3 months of the standard antiparkinsonian therapy.

Combination therapy with PM-40 had a normalizing effect on immune parameters in PD patients. This

treatment was followed by an increase in the number of T cells ($CD3^+$, 64.5±1.0%; $CD4^+$, 36.7±0.9%) and B cells ($CD20^+$, 9.7±0.7%). We revealed a decrease in the number of natural killer cells ($CD16^+$, 11.0±1.7%), macrophages ($CD11b^+$, 15.7±1.9%; $CD18^+$, 68.6±3.7%), cells expressing the IL-2 receptor ligand ($CD25^+$, 2.3±0.5%), and cells expressing the apoptotic antigen ($CD95^+$, 24.5±3.0%).

Before PM-40 treatment, the contents of IL-6, TNF-α, and IFN-γ in the blood of PD patients were 39.2±3.0, 3.84±1.70, and 0.09±0.04 pg/ml, respectively. The concentrations of IL-6 and TNF-α decreased (7.2±1.2 and 0.07±0.03 pg/ml, respectively), while the content of IFN-γ increased (4.1±2.3 pg/ml) after PM-40 therapy.

Hence, immune parameters return to normal after treatment with PM-40. This drug prevents negative activation of immune cells. Therefore, PM-40 has immunomodulatory activity (*i.e.*, stimulates the release of interferon and exhibits the anti-inflammatory properties).

PD patients were examined after 3 months of antiparkinsonian therapy. Cortisol concentration in these patients (893.5±86.3 nmol/ml) was higher compared to the normal physiological level (130-750 nmol/ml) and age control group (735±46 nmol/ml). Combination therapy with PM-40 was followed by a decrease in cortisol level to the upper limit of normal (718.5±83.8 nmol/ml).

The relief of clinical symptoms and normalization of immunobiological parameters in PD patients are mainly associated with a wide range of effects of PM-40. PM-40 has the neuroprotective, antimutagenic, antioxidant, hormone-modulating, and immunomodulatory (anti-inflammatory and interferon-inducing) properties. It can be hypothesized that the neuroprotective effect of PM-40 contributes to the recovery of dopamine synthesis by reversibly damaged neurons. Hence, PM-40 improves the efficacy of combination therapy for PD. Previous experiments showed that PM-40 decreases the content of caspase-3 and degree of DNA fragmentation in the striatum of mice with MPTP-induced parkinsonism (by 3.7 times and 19%, respectively). This action was probably related to the suppression of neuronal apoptosis [3].

Our results indicate that combination therapy with PM-40 and standard antiparkinsonian drugs has a positive clinical effect, which is manifested in the improvement of the quality of life in patients. The dose of L-DOPA-containing drugs can be reduced under these conditions. It should be emphasized that long-term treatment with these drugs causes serious side effects and aggravates the symptoms of patient's disability. PM-40 potentiates the therapeutic action and reduces the side effect of standard drugs. PM-40 has a combined effect on the pathogenetic mechanisms and various components of the pathological system in PD patients. These data should be taken into account in the development of new neuroprotective drugs to increase the efficacy of CPT.

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