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# Impact of L-DOPA treatment of patients with Parkinson's disease on mononuclear subsets and phagocytosis in the peripheral blood

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**Abstract**

*Until now, the question as to the role of immunological mechanisms in neuronal death of extrapyramidal cell systems in Parkinson's disease (PD) has not been fully resolved.*
*One of the approaches includes examination of circulating blood cells. In our studies on 24 PD patients, peripheral blood was studied before and after medication with L-DOPA compounds. Patients with PD demonstrated an increase in lymphocyte CD95/CD3 ratio as well as a considerable number of cells dead due to apoptotic processes. After treatment with L-DOPA, both the CD95/CD3 ratio, assumed to represent an antigen marker characteristic of apoptotic cells, and the number of cells dead due to apoptotic processes were found to be decreased. Thus, the findings indicate that levodopa treatment in PD has an impact on apoptotic processes and this should be taken into consideration as a positive event in the pathomechanism precipitated by this treatment.*

***Key words:*** *L-DOPA, Parkinson's disease, mononuclear subset, apoptosis, phagocytosis.*

## Introduction

Parkinson's disease (PD) is a neurodegenerative disorder of middle- and old-aged humans. The overwhelming neurochemical dysfunction involves loss of dopamine in nigrostriatal and in other neurotransmitter systems, including noradrenergic and cholinergic ones, accompanied by persistent equilibrium in the immune system [1]. Numerous reports, starting with the important paper of McGeer *et al*. [11], have indicated that an inflammatory process in the brain in the form of microglial activation, acts as a factor which triggers the degenerative process. However, it is not clear whether the microglial activation is the cause of cellular lesions and cell death or it is only a secondary reaction, developing in the course of neuronal degeneration in the brain [12].

An impact of immune factors on the pathomechanism of PD has been discussed for years [4-9,13,14].

The basic, but so far unresolved question involves the role of immunological mechanisms in neuronal death within the extrapyramidal cell system. One possible approach in respective studies involves examination of circulating blood cells and humoral cytokines in PD patients. The problem is not new, but so far

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the question whether medication with L-DOPA compounds has any important impact on immunological events in peripheral blood of PD patients remains unresolved. Due to its therapeutic implications the question seems to be of general importance.

## Material and methods

The study group comprised 24 patients (10 females and 14 males) with early diagnosed PD. The mean age of patients was 67 ± 8.4 years (ranging from 51 to 84 years). The duration of the disease ranged from 1 to 5 years. The patients have never before been treated with L-DOPA preparations. Peripheral blood was taken before the onset of treatment and after medication with L-DOPA compounds. The duration of treatment was 207 ± 12 days, ranging from 181 to 229 days. The patients were treated with 300 to 800 mg of an L-DOPA compound per day. The following drugs were used: Madopar HBS (100 mg of L-DOPA) and Madopar 62.5 (50 mg of L-DOPA). The dosage was adjusted to the response in the course of hospital observation. From that time on, the patients were kept on a stable dose of L-DOPA. Some details of the applied treatment are presented in Table I.

The control group consisted of 29 persons (14 females and 15 males) aged from 46 to 86 years (mean 64.4 ± 9.4 years), without any evident disease of the central nervous system or extrapyramidal syndrome.

Lymphocyte subsets were analysed by flow cytometry with the aid of specific monoclonal antibodies purchased from the Becton Dickinson Company (San Jose, LA). The evaluation was performed using the Cytoson Absolute (Ortho Diagnostic System) at the wavelength of 480 nm. The obtained relative results were analysed using the Immuno Count II program. The statistical analysis of results was performed using the Mann-Whitney *U* test to determine significance of differences between initial values and those after L-DOPA treatment.

## Results

The studied group of patients with PD demonstrated an increase in the CD95/CD3 lymphocyte subset, assumed to represent a marker of apoptotic cells, and an increase in the number of Apo(+) Pi(+) positive cells, representing cells killed by apoptotic processes in their late phase (Table II).

After treatment with L-DOPA, the immunomarkers of peripheral blood cells demonstrated a relative increase in CD16 lymphocytes, while the percentage of CD19 lymphocytes, and the ratios of CD4/CD8 and CD95/CD3 were decreased (Table III).

The number of granulocytes dead due to the apoptotic process in its late phase significantly declined in treated patients (Table III).

## Discussion

Immune abnormalities were documented in PD, including important occurrence of autoantibodies against selected neuronal structures with high numbers of activated microglial cells in the extrapyramidal system, predominantly in the substantia nigra. Activated microglia may participate in the death of neurons and reflect activity of toxic substances. Neuroinflammation in PD also seems to be connected with peripheral immune reactions. Changes in lymphocyte subsets in cerebrospinal fluid and in peripheral blood were also observed in PD, accompanied by enhanced synthesis of immunoglobulins and cytokines and augmented production of acute phase proteins. The alterations in mononuclear cell numbers in peripheral blood of PD patients have already been extensively discussed but have only partially been related to age-matched control groups. The later comparison is essential because of the senescence-accompanying increase in CD4(+) CD25(+) regulatory cells, noted in parallel to a general decline of immune responses. Hence, observations indicating that the involvement

**Table I.** Medication of Parkinson's patients with L-DOPA

| L-DOPA dose in mg per day | 300 | 400 | 450 | 600 | 800 |
|---|---|---|---|---|---|
| Number of patients | Total 1 (F – 1, M – 0) | Total 2 (F – 0, M – 2) | Total 3 (F – 1, M – 2) | Total 13 (F – 6, M – 7) | Total 5 (F – 2, M – 3) |

*F – female, M – male*

*The L-DOPA compounds were: Madopar HBS – 1 tablet 100 mg of L-DOPA and Madopar 62.5 mg containing 50 mg of L-DOPA+--.*

**Table II.** Markers of phagocytosis and apoptosis in patients with Parkinson's disease before and after L-DOPA treatment

| | Controls $n = 29$ | | Parkinson's disease $n = 24$ | | | Parkinson's disease after L-DOPA treatment $n = 24$ | | |
|---|---|---|---|---|---|---|---|---|
| | mean ± SD | median (min-max) | mean ± SD | median (min-max) | *P* | mean ± SD | median (min-max) | *P* |
| Phagotest difference in % | 71.5 ± 5.7 | 72 (53-80) | 66.6 ± 10.7 | 68 (44-87) | **0.035*** | 70.8 ± 8.3 | 70 (55-86) | **0.043*** |
| Lymph. Apo(–)PI(–) | 89.54 ± 3.27 | 90.12 (83.09-95.52) | 88.73 ± 4.99 | 90.08 (75.48-95.97) | 0.852 | 90.24 ± 2.75 | 90.80 (83.23-94.37) | 0.391 |
| Lymph. Apo(–)PI(+) | 5.48 ± 2.79 | 4.92 (2.24-14.58) | 4.61 ± 1.50 | 4.74 (1.67-7.82) | 0.541 | 4.66 ± 2.00 | 4.31 (1.09-10.58) | 0.864 |
| Lymph. Apo(+)PI(–) | 2.37 ± 1.31 | 2.00 (0.79-5.08) | 2.80 ± 2.22 | 2.19 (0.52-9.45) | 0.677 | 2.12 ± 1.17 | 1.89 (0.48-6.43) | 0.097 |
| Lymph. Apo(+)PI(+) | 2.61 ± 1.15 | 2.70 (0.69-4.94) | 3.94 ± 3.21 | 3.13 (1.24-12.56) | 0.141 | 3.03 ± 1.49 | 2.93 (0.33-6.00) | 0.338 |
| Granulocytes Apo(–)PI(–) | 56.55 ± 8.59 | 58.62 (30.59-66.97) | 48.26 ± 9.91 | 47.10 (29.30-66.46) | **0.001*** | 51.84 ± 9.62 | 50.48 (35.65-71.16) | **0.021*** |
| Granulocytes Apo(–)PI(+) | 4.98 ± 3.42 | 4.59 (0.44-12.95) | 4.49 ± 2.16 | 4.29 (0.71-8.51) | 0.951 | 4.32 ± 2.02 | 4.58 (0.91-7.41) | 0.841 |
| Granulocytes Apo(+)PI(–) | 12.69 ± 6.16 | 11.74 (3.71-30.00) | 13.73 ± 8.41 | 10.89 (3.60-30.00) | 0.922 | 13.81 ± 8.50 | 11.87 (3.24-34.54) | 0.775 |
| Granulocytes Apo(+)PI(+) | 25.71 ± 5.97 | 23.74 (18.05-39.23) | 33.51 ± 8.48 | 32.58 (21.45-48.57) | **< 0.001*** | 29.20 ± 7.92 | 27.17 (18.27-44.45) | **< 0.001*** |

**Significant difference*

*Description. Phagocytosis difference in % between internal control (in temperature of experiment 4°C and in physiological temperature 37°C)*

*Apo(-)PI(-) – living cells, Apo(-)PI(+) – dead cells by the necrotic process, Apo(+)PI(-) – dead cells by the apoptotic process (early phase of apoptosis), Apo(+)PI(+) – dead cells by the apoptotic process (late phase of apoptosis)*

**Table III.** Surface immunomarkers of peripheral blood lymphocytes in patients with Parkinson's disease in relative values (%)

| | Controls *n* = 29 | | Parkinson's disease *n* = 24 | | | Parkinson's disease after L-DOPA treatment *n* = 24 | | |
|---|---|---|---|---|---|---|---|---|
| | mean ± SD | median (min-max) | mean ± SD | median (min-max) | *P* | mean ± SD | median (min-max) | *P* |
| CD3% | 78.8 ± 4.5 | 79.5 (68.8-84.8) | 79.8 ± 4.3 | 79.7 (69.6-85.9) | 0.495 | 79.4 ± 4.8 | 79.2 (70.9-86.8) | 0.721 |
| CD4% | 48.7 ± 7.3 | 47.6 (34.7-63.4) | 51.4 ± 6.2 | 51.4 (35.4-62.9) | 0.191 | 49.4 ± 7.2 | 49.8 (35.7-61.7) | 0.130 |
| CD8% | 29.8 ± 7.3 | 27.7 (16.8-45.6) | 28.3 ± 7.2 | 27.6 (17.5-45.9) | 0.484 | 29.1 ± 7.3 | 27.5 (20.5-52.6) | 0.338 |
| CD16% | 10.4 ± 3.7 | 10.1 (4.1-19.5) | 9.0 ± 3.5 | 9.4 (3.5-16.5) | 0.244 | 11.7 ± 3.9 | 11.1 (6.3-21.4) | **< 0.001*** |
| CD19% | 10.62 ± 3.40 | 9.9 (3.8-18.9) | 10.90 ± 3.16 | 10.1 (5.9-18.6) | 0.703 | 9.32 ± 3.35 | 8.2 (4.7-17.7) | **0.004*** |
| CD4/CD8 | 1.77 ± 0.68 | 1.9 (0.9-3.6) | 2.01 ± 0.60 | 2.1 (0.7-2.9) | 0.091 | 1.81 ± 0.55 | 1.8 (0.7-2.8) | **0.043*** |
| CD95/CD3 | 2.17 ± 1.24 | 1.7 (0.4-5.0) | 3.85 ± 2.19 | 3.5 (0.5-8.7) | **0.002*** | 2.66 ± 1.17 | 2.7 (0.3-5.3) | **0.025*** |
| CD95/CD19 | 2.73 ± 1.48 | 2.3 (0.6-7.6) | 2.50 ± 1.52 | 2.2 (0.4-6.6) | 0.553 | 2.69 ± 1.33 | 2.8 (0.5-5.4) | 0.317 |

**Significant difference*

of T4+ helper/inducer/cells may be decreased in PD seem to be controversial.

The results of our studies do not demonstrate any significant differences in CD4, CD8, CD16 and CD19 counts in peripheral blood lymphocytes of PD patients, when matched with age appropriate controls. The same pertains also to the ratio of CD4/CD8 lymphocytes.

The results of our studies, demonstrating in PD a significant increase of lymphocyte subsets CD95/CD3, the markers of an antigen located on cells affected by apoptotic processes, can serve as a valid argument for the statement that dopaminergic cell death is influenced by an innate immune system, which has already been documented in the experimental mouse model of PD [2]. Our finding, demonstrating an increased number of granulocytes dead by apoptosis in its late phase, is consistent with the data.

Enhanced apoptosis in peripheral lymphocytes of PD patients was also shown by Calopa *et al.* [3]. According to the study, spontaneous and activation-induced apoptosis of CD4(+) T cell subsets was increased, thus pointing to enhanced susceptibility to apoptosis with Fas involvement. Invading T lymphocytes contributing to neural cell death via the Fas/Fasl pathway have also been shown in the experimental mouse model of PD [2].

As to the effect of L-DOPA treatment on immunological processes in PD, there is only very scanty information available. Fiszer *et al.* [6] reported that treatment with levodopa induced a selective effect on the immunological system of patients with PD as indicated by an increase in the synthesis of interleukin-1 and in IgM and IgA levels in blood plasma. Bas *et al.* [1] found that lymphocyte populations are not dependent on levodopa treatment in patients with PD.

In our study material we observed that after treatment with L-DOPA an appreciable increase had occurred in the relative amount of CD16 and CD19 lymphocytes, accompanied by a decrease in the CD4/CD8 ratio and in the number of lymphocytes carrying CD95/CD3 antigens. The CD95 antigen is characteristic of apoptotic cells within T/CD3(+) lymphocyte population. The observations are in line with our results, showing that after medication with levodopa the number of living Apo(–)PI(–) granulocytes had increased in parallel with a significant decrease in the number of dead granulocytes, resulting from an enhanced apoptotic process in the late phase of apoptosis.

Apoptosis-related proteins are elevated in the striatum of patients with PD, and this indicates that neuronal death in this disease involves an apoptotic

process [10]. Indirect signs of apoptosis were observed in brain neurons and lymphocytes of peripheral blood. T cells manifested increased susceptibility to apoptosis with Fas involvement [3]. Therefore, our results confirming the impact of levodopa treatment on apoptotic processes in the peripheral blood of patients with PD supplement the understanding of L-DOPA's positive effect in PD patients.

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