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Oxidative DNA Damage and the Level of Biothiols, and L-Dopa Therapy in Parkinson's Disease

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1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurological disorder characterized by resting tremor, rigidity, and bradykinesia, affecting at least 1% of individuals above the age of 65 years. Parkinson's disease is a result of degeneration of the dopamine-producing neurons of the *substantia nigra*. Available therapies in PD will only improve the symptoms but not halt progression of disease. The most effective treatment for PD patients is therapy with L-3,4-dihydroxy-phenylalanine (L-dopa) (Olanow, 2008). As indicated in literature reports, L-dopa therapy leads to motor fluctuations and disabling involuntary movements called L-dopa-induced dyskinesia (Carta et al., 2006; Obeso et al., 2008). Literature reports indicate also that long-term administration of L-dopa in PD patients may not only alter arginine (Arg) levels but may also lead to increased concentrations of homocysteine (Hcy), the factor responsible for development of atherosclerosis and dysfunction of nigral endothelial cells (Muller et al., 1999).
Methylenetetrahydrofolate reductase (MTHFR) represents enzyme involved in remethylation of Hcy to methionine (Met). The C667T transition in *MTHFR* results in Ala>Val substitution in position 226 and, as a consequence, in 50 % decrease in the enzyme activity, and thus in an increased concentration of Hcy (Frosst et al., 1995). The study of Yasui et al. (2000) indicated that the TT genotype might be linked to pathogenesis of PD, particularly when the level of folates is low. Moreover, L-dopa metabolism via O-methylation by catechol-O-methyl-transferase (COMT) using S-adenosyl-L-methionine (SAM) leads to increase Hcy levels, hyper-Hcy (O'Suilleabhain et al., 2004a). A percentage 10-30% of PD patients exhibits hyper-Hcy. Hyper-Hcy in PD has been associated with affective and cognitive impairment, dementia, dyskinesia, and vascular disease (O'Suilleabhain et al., 2004b; Rogers et al., 2003; Zoccolella et al., 2006, 2009).
The exact mechanism of development and progression of PD pathology is not clear. It is known, that a complex interplay of multiple environmental and genetic factors has been involved in pathogenesis of PD and it is possibly that PD represents rather a syndrome but not a single disorder. Moreover, is likely that in pathogenesis of PD there are several mechanisms involved, such as: oxidative stress, mitochondrial dysfunction, DNA damage, protein aggregation, neuroinflammation, excitotoxicity, apoptosis and loss of trophic factors. The most probably is that all factors are represented targets for PD therapy.

2. 8-Oxo-2'-deoxyguanosine and L-dopa treatment in Parkinson's disease

Oxidative stress and excitotoxicity seems to play a pivotal role in pathogenesis of few major neurodegenerative diseases e.g. Alzheimer's disease (AD) and PD. The study of Jenner (2003) indicates that oxidative stress in the brain of PD patients may leads to formed reactive forms of oxygen (RFO). In the course of PD, RFO activate processes leading to the damage of DNA, proteins and lipids, and to a low level of antioxidants (Blake et al., 1997; Kikuchi et al., 2002). Moreover, in the patients suffering from PD, dopamine level-controlled deposition of ubiquitin- and α-synuclein-positive inclusion bodies (Lewy's bodies) takes place in the cytoplasm of dopaminergic neurons (Spillantini et al., 1997). Deposition of pathological proteins in brains of patients affected by the neurodegenerative diseases, result in pronounced neurotoxic effects on the central nervous system (CNS). In PD, augmented expression of α-synuclein may intensify oxidative stress (Hsu et al., 2000). Bergman et al. (1998) demonstrated that in the PD patients, dopaminergic neurons undergo oxidative damage of the compact portion of *substantia nigra* and dopamine levels decrease in putamen, a region of caudate nucleus. Moreover, ferrous ions released from damaged *substantia nigra* may provide an important substrate for oxidative reactions and for production of RFO (Jenner, 2003).

In PD, oxidative stress follows accumulation of the degradation products in the gray matter compact part of mesencephalon, and is accompanied not only by a high level of ferrous ions, and by decreased level of glutathione, malfunction of the respiratory chain complex I (Jenner, 2003; Schapira et al., 1990; Sian et al., 1994), and excessive oxidation processes, especially in patients treated with L-dopa (Spencer et al., 1994).

L-Dopa after oral intake undergoes metabolism, including oxidative metabolism of dopamine, and auto-oxidation, and is transported across the blood-brain barrier. Only less than 5% of an oral dose of L-dopa after took delivered to the brain. Remain plasmatic levels of L-dopa undergoes peripheral oxidative metabolism and may generate ROS. Likely peripheral oxidation status in PD might be affected by L-dopa therapy (Cornetta et al., 2009).

Some studies (e.g. Cornetta et al., 2009) suggest a toxic effect of L-dopa on neuronal cell *in vitro*, while *in vivo* studies in animal models are contradictory. However, in patients with PD some authors indicated on positive correlation between oxidative stress and L-dopa therapy (Florczak et al., 2008; Migliore at al., 2002), but there are also negative correlation between oxidative stress and L-dopa dosage in peripheral blood lymphocytes (in nine patients with PD et paper of Cornetta et al., 2009; Prigione et al., 2006).

As indicated by literature reports, interaction of reactive oxygen with nucleic acids leads to oxidation of guanine and formation of 8-oxo-2'-deoxyguanosine (8-oxo2dG). Oxidative modification of guanine at C8 position may take place either in nucleic acids or free cellular nucleosides and nucleotides, ready to be incorporated to newly synthesized DNA chains. Incorporation of the modified nucleotide to DNA may results in mutations due to pairing of 8-oxoguanosine with cytosine and adenosine. In the course of pairing with adenosine, 8-oxoguanosine induces GC→AT transversions (Hirano, 2008). 8-Oxoguanina or its nucleoside, 8-oxo2dG there are though to represent markers of oxidative DNA damage.

Augmented levels of 8-oxo2dG were demonstrated in brain and in lymphocytes of patients with PD (Alam et al., 1997; Dorszewska et al., 2007; Florczak et al., 2008; Kikuchi et al., 2002; Zhang et al., 1999). This indicates a gradual increase of nucleic acid damage during development of this disease, and high level of oxidized guanine in DNA is considered a risk factor for senescence and neurodegenerative diseases (e.g. PD).

The contribution of L-dopa therapy to oxidative damage and apoptosis in peripheral cells in PD patients is not clear, and is still debated.

2.1 Influence of L-dopa treatment on the level of 8-oxo-2dG in peripheral blood lymphocytes of Parkinson's disease patients

The aim of the study was to estimate the degree of oxidative damage to DNA (marker: 8-oxo2dG) in PD patients before and during treatment with L-Dopa, and in controls.

2.1.1 Patients

The studies were conducted on 98 patients with PD, including 37 women and 61 men aging 34-81 years (mean age: 60.8±10.7 years). Among the patients with PD, 27 patients (9 women and 18 men) awaited L-dopa treatment (patients' age: 34-79 years) and the remaining 71 individuals, 28 women and 43 men (patients' age: 35-81 years) were treated with L-dopa preparations in daily doses (up to 5 years treatment to 500 mg/day, 5-10 year treatment 500-800 mg/day, and over 10 year treatment 800-1500 mg/day).

Control group included 50 individuals, 34 women and 16 men, 22-76 years of age (mean age: 44.6±16.2 years).

Patients with PD were diagnosed using the criteria of UK Parkinson's Disease Society Brain Bank (Litvan et al., 2003), however stage of disease according to the scale of Hoehn and Yahr.

None of the control subjects had verifiable symptoms of dementia or any other neurological disorders and smoking, and drinking habits.

A Local Ethical Committee approved the study and the written consent of all patients or their caregivers was obtained.

2.1.2 Determination of 8-oxo2dG

Isolation of DNA. DNA was isolated from peripheral blood lymphocytes by fivefold centrifugation in a lytic buffer, containing 155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4, in the presence of buffer containing 75 mM NaCl, 9 mM Na_2EDTA , pH 8.0, and sodium dodecyl sulfate and proteinase K (Sigma, St. Louis, MO). Subsequently, NaCl was added, the lysate was centrifuged, and DNA present in the upper layer was precipitated with 98% ethanol.

Enzymatic hydrolysis of DNA to nucleosides. DNA was hydrolyzed to nucleosides using P_1 nuclease (Sigma), for 2 h at 37^{o}C in 10 mM NaOAc, pH 4.5. The solution was buffered with 100 mM Tris-HCl, pH 7.5. Subsequently, the DNA was hydrolyzed with alkaline phosphatase (1U/µl; Roche, Germany) for 1 h at 37 ^{o}C and the obtained nucleosides mixture was applied to high-pressure liquid chromatography system with both electrochemical and UV detection (HPLC/EC/UV).

Estimation of 8-oxo2dG. To determine 8-oxo2dG level, the nucleosides mixture was applied to the HPLC/UV system (P580A; Dionex, Germany) coupled to an electrochemical detector (CoulArray 5600; ESA, USA). Nucleosides were separated in a Termo Hypersil BDS C18 (250 x 4.6 x 5µm) column (Germany). The system was controlled, and the data were collected and processed using Chromeleon software (Dionex, Germany). The results were expressed as a ratio of oxidized nucleosides in the form of 8-oxo2dG to unmodified 2'dG (Olsen et al., 1999).

2.1.3 Results

In the patients with PD (Table 1), the levels of 8-oxo2dG in peripheral blood lymphocytes were significantly increased ($p<0.05$), as compared to the controls.

Parameter	Controls (22-76 years)	Patients with PD (34-81 years)
8-oxo2dG	13.7 ± 7.6	21.8 ± 17.8*

Table 1. Levels of DNA oxidative damage (8-oxo2dG/dG x 10^{-5}) in the PD patients and in control group. Results are expressed as a means ± SD. The nonparametric of Mann-Whitney test for unlinked variables was used. Differences significant at *$p<0.05$, as compared to the controls.

In the PD patients (Table 2) disease progress from stage I to IV (according to the scale of Hoehn and Yahr) resulted in higher level of 8-oxo2dG in DNA ($p<0.05$) also observed between stages I and III, and a tendency to further decrease in stage IV.

Parameter	Stage I (35-79 years)	Stage II (34-81 years)	Stage III (46-81 years)	Stage IV (56-78 years)
8-oxo2dG	17.4 ± 16.9	20.5 ± 14.2	25.2 ± 22.7	23.2 ± 12.8*

Table 2. Levels of oxidative DNA damage (8-oxo2dG/dG x 10^{-5}), as related to the stage of the PD according to the scale of Hoehn and Yahr. Results are expressed as means ± SD. The nonparametric of Mann-Whitney test for unlinked variables was used. Differences significant at *$p<0.05$, between stages I and IV of PD.

Pharmacotherapy with L-dopa (Table 3) affected the level of 8-oxo2dG ($p<0.01$), as compared to the healthy controls.

Parameter	Controls (22-76 years)	Patients with PD L-dopa (-) (34-79 years)	Patients with PD L-dopa (+) (35-81 years)
8-oxo2dG	13.7 ± 7.6	19.3 ± 17.3	22.6 ± 18.0**

Table 3. Levels of oxidative DNA damage (8-oxo2dG/dG x 10^{-5}), as related to pharmacotherapy with L-dopa (+) in the patients with PD. Results are expressed as means ± SD. The nonparametric of Mann-Whitney test for unlinked variables was used. Differences significant at **$p<0.01$, as compared to the controls.

Our results indicated that, L-dopa can modify the level of oxidative DNA damage (8-oxo2dG) in the peripheral blood cells of PD patients. On the other hand, it is interesting that in PD a significant increase in DNA damage has been observed in the IVth stage of the disease development (according to Hoehn and Yahr), even so 8-oxo2dG levels are increased between the stages I and III of the disease evolution. It seems that in PD the reason for increasing levels of oxidative process altered nucleic acids is thought to involve overproduction of free radicals as well as decreased levels of enzymatic and non-enzymatic antioxidants and less effective repair mechanisms. In AD patients have been found to contain lowered activity of specific 8-oxoguanine glycosylase 1, OGG1, and more oxidative DNA damage which might induce of apoptosis (Dorszewska et al., 2005, 2009a, 2010).

It seems that analysis of the level of oxidative stress (8-oxo2dG) may be represented targets for diagnosis of PD and therapy in future.

2.2 Influence of L-dopa treatment on the level of apoptotic factors in peripheral blood lymphocytes of Parkinson's disease patients

At the neuropathological studies, PD is mainly characterized by neuronal intracellular inclusions named Lewy's bodies with α-synuclein. These inclusions are now known to be comprised of filamentous polymers of α-synuclein, which may generate oxidative stress in the brain of PD patients. It could results from several mechanisms, such as depletion of antioxidants, defects in mitochondrial electron transport, neurotoxin exposure, and excessive oxidation of dopamine in the patients given L-dopa. Conway et al. (2001) showed that dopamine or L-dopa inhibits the fibrillization of α-synuclein filaments by stabilization of their structure.

However Alves Da Costa et al. (2002) showed that α-synuclein drastically lowered caspase-3 activity and p53 protein expression, and transcriptional activity, proteins controlled the apoptotic cascade. Blandini et al. (2004), Dorszewska et al. (2009b) and Iwashita (2004) showed that, apoptotic proteins such as: Bcl-2 family proteins and PARP are involved in the pathogenesis of PD as well.

The aim of the study was to estimate the levels of p53, and PARP proteins, and 85 kDa fragment, and two Bcl-2 family proteins: Bcl-2 and Bax in peripheral lymphocytes of patients with PD and in control group. The attention was also paid to L-dopa pharmacotherapy in PD.

2.2.1 Patients

The studies were conducted on 45 patients with PD, among their 22 patients, including 9 women and 13 men, aging 41-79 years (mean age: 58.0±10.7 years) awaited L-dopa treatment and 23 patients, including 11 women and 12 men aging 45-81 years (mean age: 68.0±8.6 years) were treated with L-dopa preparations in daily doses (up to 5 years treatment to 500 mg/day, 5-10 year treatment 500-800 mg/day, and over 10 year treatment 800-1500 mg/day).

The control group included 27 individuals, 19 women and 8 men, 35-73 years of age (mean age: 54.0±10.7 years).

Patients with PD were diagnosed using the criteria of UK Parkinson's Disease Society Brain Bank (Litvan et al., 2003), however stage of disease according to the scale of Hoehn and Yahr.

None of the control subjects had verifiable symptoms of dementia or any other neurological disorders and smoking, and drinking habits.

A Local Ethical Committee approved the study and the written consent of all patients or their caregivers was obtained.

2.2.2 Estimation of p53, Bax, Bcl-2, PARP proteins and 85-kDa subunit

Isolation of proteins. Blood was gradiated onto gradisol L at a 1:1 ratio and centrifuged, followed by collection of the interphase which was then rinsed in PBS buffer (0.9% NaCl in phosphate buffer) and centrifuged. The obtained lymphocyte precipitate was rinsed with radioimmunoprotein assay (RIPA) buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1% IGEPAL CA-630, 0.05% SDS, and 1% sodium deoxycholate), supplemented with a

protease inhibitor cocktail (Sigma) and homogenized in a mixture of RIPA with protease inhibitor cocktail (16:1) and 0.5 µl PSMF (Sigma) in isopropanol (10 mg/100 µl), centrifuged, and the obtained supernatant underwent further analysis (Ohnishi et al., 1996).

Western Blot. The Bax and Bcl-2 proteins were analyzed in 12% and p53, and PARP proteins were analyzed in 7.5% polyacrylamide gel. Equivalent amounts of protein (30 µg protein/lane) were loaded to the wells. The gel-separated proteins were electrotransferred to nitrocellulose filter in a semidry Western Blot analysis apparatus (Apelex, France). To estimate the levels of the PARP protein, the filters were exposed first to an anti-PARP monoclonal antibody (G-2-10, IgG, 0.05 ml, Sigma, USA), diluted 1:2000, while the p53, Bax, Bcl-2 proteins were identified using anti-p53 (IgG-2a, 200 µg/1.0 ml; Santa Cruz, USA), anti-Bax (IgG-2b, 200 µg/1.0 ml; Santa Cruz, USA) and anti-Bcl-2 (IgG-1, 200 µg/1.0 ml; Santa Cruz, USA) mouse monoclonal antibody, respectively, diluted 1:500.

Subsequently, individual sheets of nitrocellulose filter were incubated with the second antibody, goad antimouse IgG-HRP (200 µg/0.5 ml; Santa Cruz, USA) at a dilution of 1:2000. To stain immunoreactive bands, peroxidase BMB was added (BM blue POD substrate precipitation; Roche, Germany). The surface area of the immunoreactive bands was registered using a densitometer (GS-710; Bio-Rad, Hercules, CA) in the Quantity One System.

2.2.3 Results

The studies disclosed significant decreased levels of apoptotic proteins (p53, Bax:Bcl-2, PARP, $p<0.01$; Bax, Bcl-2, $p<0.001$) in PD as compared to the controls (Table 4). Decreased level of apoptotic proteins in PD patients probably was result influence of α–synuclein on lower p53 protein expression and caspase-3 activity.

Parameter	Controls 35-73 years of age	PD patients 41-81 years of age
p53	0.52 ± 0.37	0.25 ± 0.14**
Bax	0.60 ± 0.50	0.13 ± 0.07***
Bcl-2	1.20 ± 0.77	0.15 ± 0.08***
Bax/Bcl-2	1.46 ± 3.77	1.13 ± 0.83**
PARP	2.12 ± 0.83	1.61 ± 1.12**
85-kDa	0.42 ± 0.80	0.41 ± 0.31

Table 4. Level of p53, Bax, Bcl-2, PARP proteins and of 85-kDa protein subunit in peripheral blood lymphocytes in PD patients and in the control group. Apoptotic proteins represent % of area of immunoreactivity bonds. Results are expressed as a means ± SD. The nonparametric test of Mann-Whitney was used. Differences significant at **$p<0.01$; ***$p<0.001$ as compared to the controls.

Simultaneously, in PD patients treated with L-dopa preparations (Table 5) levels of p53, Bax, Bcl-2 proteins increased unsignificant as compared with untreatment patients. In the PD patients treated with L-dopa significant increased only the levels of PARP protein ($p<0.001$ as compared to patients not treated with L-dopa) and 85-kDa fragment ($p<0.01$ as compared to patients untreated with L-dopa).

Parameter	Controls 35-73 years of age	PD patients L-dopa (-) 41-79 years of age	PD patients L-dopa (+) 45-81 years of age
p53	0.52 ± 0.37	0.19 ± 0.11**	0.30 ± 0.15
Bax	0.60 ± 0.50	0.12 ± 0.06***	0.14 ± 0.08***
Bcl-2	1.20 ± 0.77	0.15 ± 0.06***	0.16 ± 0.10***
Bax/Bcl-2	1.46 ± 3.77	1.00 ± 0.64	1.26 ± 0.97*
PARP	2.12 ± 0.83	0.82 ± 0.36***	2.36 ± 1.08(***)
85-kDa	0.42 ± 0.80	0.22 ± 0.13	0.58 ± 0.32***(**)

Table 5. Level of p53, Bax, Bcl-2, PARP proteins and of 85-kDa protein subunit in peripheral blood lymphocytes in PD patients untreatment L-dopa (-) and treatment L-dopa (+), and in the control group. Apoptotic proteins represent % of area of immunoreactivity bonds. Results are expressed as a means ± SD. The nonparametric test of Kruskal-Wallis was used. Differences significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the controls, and (**)p<0.01; (***)p<0.001 between PD patients untreatment L-dopa (-) and treatment L-dopa (+).

However, (Table 6) long-term (more than 5 years) therapy of L-dopa in PD patients probably leads to apoptosis, because elevated levels of Bax:Bcl-2 ratio (p<0.05 as compared to the controls) and 85-kDa fragment (p<0.05 as compared to the controls).

Parameter	Controls	PD patients L-dopa < 5 years	PD patients L-dopa > 5 years
p53	0.52 ± 0.37	0.30 ± 0.13	0.30 ± 0.16
Bax	0.60 ± 0.50	0.14 ± 0.09**	0.15 ± 0.08***
Bcl-2	1.20 ± 0.77	0.19 ± 0.08*	0.15 ± 0.11***
Bax/Bcl-2	1.46 ± 3.77	0.85 ± 0.69	1.40 ± 1.03*
PARP	2.12 ± 0.83	2.03 ± 1.00	2.48± 1.12
85-kDa	0.42 ± 0.80	0.44 ± 0.20	0.64 ± 0.35**

Table 6. Level of p53, Bax, Bcl-2, PARP proteins and of 85-kDa protein subunit in peripheral blood lymphocytes in PD patients treatment L-dopa less and more than 5 years, and in the control group. Apoptotic proteins represent % of area of immunoreactivity bonds. Results are expressed as a means ± SD. The nonparametric test of Kruskal-Wallis was used. Differences significant at *p<0.05; **p<0.01; ***p<0.001 as compared to the controls.

It seems that pharmacological treatment of PD patients with L-dopa has a major role in modulating of levels in lymphocytes of some apoptotic proteins, important for this process. Further investigation is thus requisite to analysis expression and mutations of genes encoding proteins important for effective repair and/or apoptosis in PD patients treatment with L-dopa.

3. Homocysteine and asymmetric dimethylarginine and L-dopa treatment in Parkinson's disease

Elevated Hcy level is a risk factor for vascular diseases, cognitive impairment and dementia, and neurodegenerative diseases (e.g. PD). It is also known that vascular dementia and cognitive impairment worsen the prognosis of PD patients, and it is important to minimize

the risk of their occurrence as much as possible. Gorell et al. (1994) indicated that patients with PD have shown an increased risk for cardiovascular disease and stroke. In PD not only Hcy, but also cysteine (Cys), product of Hcy metabolism may promote pathological alterations such as: atherosclerosis and thrombogenesis (Muller, 2008).

3.1 Metabolism of biothiols

In the body, Hcy is a point of intersection of two main metabolic pathways: transsulfuration and remethylation. Under physiological conditions, around 50% of Hcy is catabolized by transsulfuration and undergoes transformation to cystathionine and then to Cys. The remaining 50% of Hcy undergoes methylation to Met (Fig. 1).

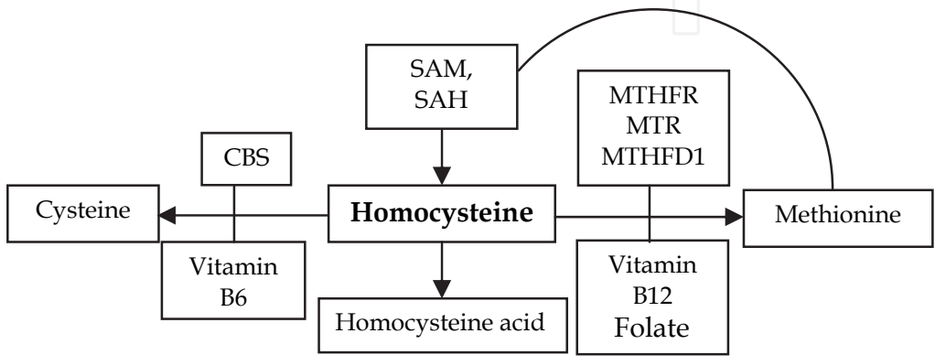


Fig. 1. Synthesis and metabolic pathways of homocysteine, CBS- cystationine β-synthase, MTHFR- 5,10-methylenetetrahydrofolate reductase, MTR- methionine synthase, MTHFD1- methylenetetrahydrofolate dehydrogenase/ methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase, SAH- S-adenosylhomocysteine, SAM- S-adenosylmethionine.

Methionine is supplied with food and its transformation to Hcy involves several steps. At the first step, Met is transformed to SAM and is then demethylated to SAH (S-adenosylhomocysteine) and hydrolyzed to Hcy. SAM is the main donor of methyl groups in many reactions. A decreased content of SAM was demonstrated in the course of PD (Cheng et al., 1997).

The level of Hcy undergoes control, depending upon concentration of its metabolites: Cys and Met. In the case of Met deficit and low concentration of SAM, most Hcy undergoes remethylation to Met, catalyzed by methionine synthase (MTR). MTR is a vitamin B12-dependent enzyme responsible for transfer of methyl groups from N-methyltetrahydrofolate to Hcy, leading to formation of Met (Jarrett et al., 1996). Mutations in the MTR gene are responsible for decreased methylcobalamine level, and result in homocysteinuria, hyperhomocysteinemia and hypomethioninemia (Watkins et al., 2002). The tri-functional enzyme, methylenetetrahydrofolate dehydrogenase/ methenyltetrahydrofolate cyclohydrolase/formyltetrahydrofolate synthetase (MTHFD1) represents another enzyme linked to transformation of Hcy to Met. Homozygotes of both *MTHFR* and *MTHFD1* are at risk of cardiovascular diseases connected with elevated levels of Hcy, or folate level-related hypoplasia of neural tube (Hol et al., 1998). However, in the literature, less numerous data are available on the involvement of MTHFD1 in the pathogenesis of degenerative diseases (Dorszewska et al., 2007).

Under normal conditions, in the presence of a positive Met balance, most of Hcy undergoes transsulfuration catalyzed by cystathionine β-synthetase (CBS), which requires derivative of vitamin B6, pyridoxal phosphate.
Homocysteine or it oxidative product, homocysteine acid are thought to exhibit its pro-oxidative activity most probably through its direct interaction with NMDA receptors (it represents an agonist of NMDA receptor). Agnati et al. (2005) have shown that Hcy may pass the blood/brain barrier and that level of plasma Hcy corresponds to Hcy concentration in the brain.

3.2 Influence of L-dopa treatment on the plasma level of biothiols in Parkinson's disease

In PD, the high Hcy concentration may augment risk of the disease through its direct toxic effect on dopaminergic neurons. Studies *in vitro* on human dopaminergic neurons have documented a significant increase in neurotoxicity accompanying high Hcy levels (Duan et al., 2002). In parallel, elevated Hcy levels in PD have been shown to carry potential for deterioration of cognitive and motoric functions, for depression and elevated risk to develop vascular diseases (Kuhn et al., 1998).
Reports of the literature (Florczak et al., 2008; Miller et al., 2003) indicate that plasma Hcy levels in PD have been affected also by pharmacotherapy with L-dopa. It is indicated that in PD patients who are initiating L-dopa therapy, Hcy elevates within six weeks to a few months after L-dopa initiation (O'Suilleabhain et al., 2004a). Study Florczak et al., 2008 indicated that the sulfuric amino acids were also affected by duration of the L-dopa pharmacotherapy. The most exposed to neurotoxic effects of Hcy have seemed to be the patients during the first 5 years L-dopa treatment while its continued administration has resulted in stably elevated Hcy level. The study of Miller et al. (1997) indicates that L-dopa may induce elevated levels of Hcy during its methylation to 3-O-methyldopa (3-OMD) with involvement of COMT (catechol O-methyltransferase) both in peripheral blood leukocytes and in nigrostriatal neurons. In the course of the reaction, COMT in presence of magnesium ions induces in parallel transition of SAM to SAH and further hydrolysis of SAH to Hcy (Fig. 2).
Elevated level of Hcy in *substantia nigra* of PD patients has been demonstrated already after 3 months of L-dopa treatment (Yasui et al., 2003). Long-term administration of L-dopa is thought to promote benign vascular lesions in patients with PD and may result in the patients in cognitive disturbances or dementia, particular at late stages of treatment with the preparation (Muller et al., 1999). On the other hand, COMT has a broad detoxification potential in human. Two compounds are currently available, entacapone peripheral and tolcapone central blocking of COMT. COMT inhibition is also under suspicion to prevent motor complications and seems that has beneficial effect on the L-dopa-related hyper-Hcy as well (Muller, 2009a; Nevrly et al., 2010). Some animal studies shown that COMT inhibition can eliminate L-dopa-induced hyper-Hcy but not all previous studies confirm it.
Study Dorszewska et al. (2007) have shown that augmented plasma levels of Hcy in PD possibly could have developed due to altered processes of Hcy remethylation to Met and transsulfuration to Cys. Simultaneuosly, in the PD patients a decreased concentration of Met has been observed, paralleled by elevated levels of Cys and lowered ratio of Met and Cys to Hcy. The demonstrated at present decrease in Met to Hcy ratio may be linked to transformation of Hcy to thiolactone in endothelial cells. According to one of more recent hypothesis, sulfonic sulfur of thiol compounds may be involved in development of Hcy-induced arteriosclerotic lesions (Toohey, 2001). At the same time, the demonstrated at

present increased plasma Cys level in PD may result from intensified release of the amino acid from proteins, due to substitution by the circulating Hcy or due to diminished transformation of Cys to glutathione, important for maintenance of redox homeostasis in the body.

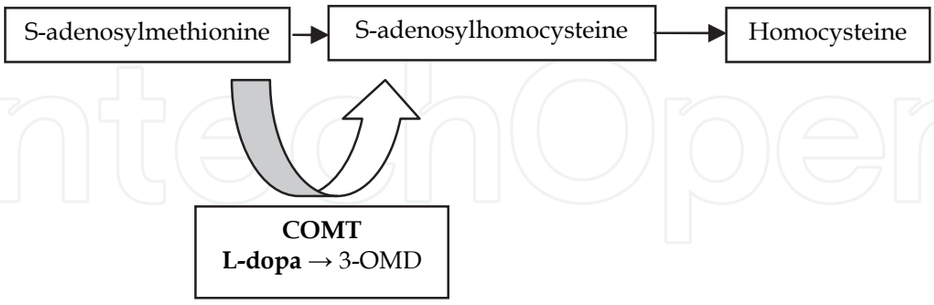


Fig. 2. COMT-mediated O-methylation of L-dopa, which results in formation of 3-methyldopa product, COMT- catecholamine-O-methyltrasferase, 3-OMD- 3-O-methyldopa.

In the literature there are studies of plasma Cys concentrations in PD patients (Dorszewska et al., 2007; Muller & Kuhn, 2009b) but there are no reported about relation between L-dope treatment and Cys status.

3.2.1 Patients

The studies were conducted on 98 patients with PD, including 37 women and 61 men aging 34-81 years (mean age: 60.8±10.7 years). Among the patients with PD, 27 patients (9 women and 18 men) awaited L-dopa treatment (patients' age: 34-79 years) and the remaining 71 individuals, 28 women and 43 men (patients' age: 35-81 years) were treated with L-dopa preparations in daily doses (up to 5 years treatment to 500 mg/day, 5-10 year treatment 500-800 mg/day, and over 10 year treatment 800-1500 mg/day).
Control group included 50 individuals, 34 women and 16 men, 22-76 years of age (mean age: 44.6±16.2 years).
Patients with PD were diagnosed using the criteria of UK Parkinson's Disease Society Brain Bank (Litvan et al., 2003), however stage of disease according to the scale of Hoehn and Yahr.
None of the control subjects had verifiable symptoms of dementia or any other neurological disorders and smoking, and drinking habits.
A Local Ethical Committee approved the study and the written consent of all patients or their caregivers was obtained.

3.2.2 Analysis of Cys concentrations

Preparation of samples. The analyzed plasma thiol compounds (Cys, Sigma, USA) were diluted with water at 2:1 ratio and reduced using 1% TCEP (Tris-(2-carboxyethyl)-phosphin-hydrochloride; Applichem, Germany) at 1:9 ratio. Subsequently, the sample was deproteinized using 1M $HClO_4$ (at 2:1 ratio) and applied to the HPLC/EC system.
Determination of thiol concentration. The samples were fed to the HPLC system (P580A; Dionex, Germany) coupled to an electrochemical detector (CoulArray 5600; ESA, USA). The analysis was performed in Termo Hypersil BDS C18 column (250 x 4.6 x 5µm) (Germany) in isocratic conditions, using the mobile phase of 0.15 M phosphate buffer, pH 2.8 supplemented with 8-10% acetonitrile for estimation of Cys (Accinni et al., 2000).

The system was controlled, and the data were collected and processed using Chromeleon software (Dionex, Germany).

3.2.3 Results

Pharmacotherapy with L-dopa of PD patients (Tables 7 and 8) leads to increase of the concentrations of metabolic product of Hcy, Cys ($p<0.01$) as compared to the controls, in the patients treated ($p<0.05$) as well as untreated ($p<0.01$) with L-dopa. Consequently, the ratio of Cys/Hcy in PD patients decreased, as compared to the controls ($p<0.05$) and to the untreated patients ($p<0.01$) as compared to treated PD patients with L-dopa.

Parameter	Controls (22-76 years)	Patients with PD (34-81 years)
Cys	220.7 ± 46.6	250.6 ± 49.6**
Cys/Hcy	19.3 ± 6.7	16.3 ± 6.5*

Table 7. Cysteine (μM) concentrations in the patients with PD and in control group. Results are expressed as a means ± SD. The nonparametric test of Mann-Whitney was used. Differences significant at *$p<0.05$, **$p<0.01$ as compared to the controls.

Parameter	Controls (22-76 years)	Patients with PD L-dopa (-) (34-79 years)	Patients with PD L-dopa (+) (35-81 years)
Cys	220.7 ± 46.6	263.6 ± 42.9**	244.7 ± 51.9*
Cys/Hcy	19.3 ± 6.7	20.7 ± 6.9	14.4 ± 5.4**(**)

Table 8. Cysteine (μM) concentrations as related to pharmacotherapy with L-dopa (+) in the patients with PD. Results are expressed as means ± SD. The nonparametric test of Mann-Whitney was used. Differences significant at *$p<0.05$, **$p<0.01$ as compared to the controls. Differences significant at (**)$p<0.01$, as compared to patients not treated with L-dopa (-).

Muller & Kohn (2009) indicated that only PD patients with an elevated level of Hcy above 15 μM showed an increase of Cys plasma level and elevated concentration of both risk factors (Hcy, Cys) may intervene in the neurodegenerative process. Present study indication that especially PD patients before L-dopa treatment showed increased level of Cys and L-dopa treatment only little decreased higher level of Cys. Increased plasma Cys level in PD may result from intensified release of the amino acid from proteins, due to substitution by the circulating Hcy or due to diminished transformation of Cys to glutathione, important for maintenance of redox homeostasis in the body. In culture of human hepatocytes 50% Cys has been demonstrated to transform into GSH (Mosharov et al., 2000). It seems also that intensity of dementive disease in particular disturbs transsulfuration of Hcy and leads to decreased levels of the agent (Cys), which provides the natural antioxidant, GSH. Homocysteine as well as Cys may serve as biomarkers for severity or progression of PD.

3.3 Influence of L-dopa treatment on the plasma level of Hcy and ADMA in Parkinson's disease patients

In the body Hcy is metabolized along two metabolic pathways, by the way of trassulfution and remethylation, involvement of SAM and SAH (Fig. 1). SAM is thought to provide the principal donor of methyl groups in numerous metabolic reactions, leading to formation of

methyl derivatives. One of the products of SAM methylation is thought to be asymmetric dimethylarginine (ADMA) (Gary & Clarke, 1998).
ADMA is an endogenous inhibitor of nitrogen oxide synthase (NOS) (Vallace et al., 1992). It arises from Arg contained in body proteins and may undergo hydrolysis to L-citruline and dimethylamine with involvement of dimethylaminohydrolase (DDAH). Homocysteine is thought to inhibit activity of DDAH (Stuhlinger et al., 2001) and might promote accumulation of ADMA that leads to a decreased production of nitrogen oxide (NO) and L-citruline from Arg with participation of NOS (Fig. 3).
NO plays an important role in control of vascular tone, in neurotransmission and in body protective mechanisms as well as in memory processes. Literature reports indicate that in PD the augmented activity of glia results in increased production of NO (McGeer et al., 1988).
ADMA is regarded to act as a risk factor for vascular diseases (Yoo & Lee, 2001). Its elevated levels were demonstrated in patients with hypercholesterolemia, hypertension, chronic heart failure and in atherosclerotic processes and during physiological aging (Kielstein et al., 2003). Role of ADMA in pathogenesis of PD is less known. Until now, in PD the levels on non-methylated substrate in biosynthesis of ADMA were examined only, and the elevated levels of Arg in cerebrospinal fluid were shown in PD patients with the decrease after L-dopa administration (Qureshi et al., 1995). Literature reports indicate also that long-term administration of L-dopa in PD patients may not only lead to increased concentrations of Hcy but may also alter Arg levels (Muller et al., 1999).

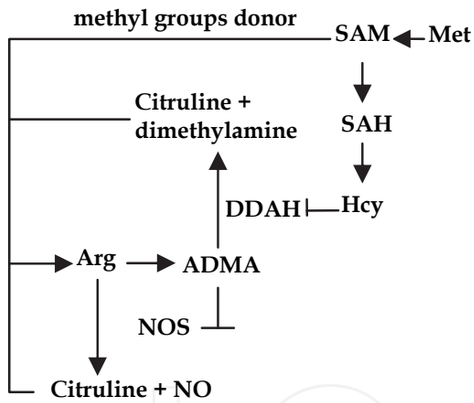


Fig. 3. The roles of methionine, homocysteine and arginine in metabolism of asymmetric dimethylarginine, Hcy- homocysteine, Met- methionine, ADMA- asymmetric dimethylarginine, Arg- arginine, NO- nitric oxide, NOS- NO synthase, DDAH- dimethylarginine dimethylaminohydrolase, SAM- S-adenosylmethionine, SAH- S-adenosylhomocysteine.

The present study was aimed at the estimation of plasma levels of Hcy and ADMA together with Met and Arg in patients with PD. The attention was also paid to developmental stages of the analyzed degenerative diseases and to L-dopa pharmacotherapy in PD.

3.3.1 Patients

The studies were conducted on 47 patients with PD, including 21 women and 26 men aging 41-86 years (mean age: 63.0±11.1 years). Among the patients with PD, 13 patients (3 women and 10 men) awaited L-dopa treatment (patients' age: 41-78 years) and the remaining 34

individuals, 18 women and 16 men (patients' age: 46-86 years) were treated with L-dopa preparations in daily doses (up to 5 years treatment to 500 mg/day, 5-10 year treatment 500-800 mg/day, and over 10 year treatment 800-1500 mg/day).
The control group included 35 individuals, 20 women and 15 men, 22-76 years of age (mean age: 45.1±16.0 years).
Patients with PD, on the other hand, were diagnosed using the criteria of UK Parkinson's Disease Society Brain Bank (Litvan et al., 2003). The stage of evolution of Parkinson's disease was defined according to the scale of Hoehn and Yahr. The tested patients represented stages I to IV of the disease evolution.
None of the control subjects had verifiable symptoms of dementia or any other neurological disorders.
A Local Ethical Committee approved the study and the written consent of all patients or their caregivers was obtained.

3.3.2 Analysis of Hcy and Met concentrations

Preparation of samples. The analyzed plasma thiol compounds (Hcy, Fluka Germany; Met, Sigma, USA) were diluted with water at 2:1 ratio and reduced using 1% TCEP (Tris-(2-carboxyethyl)-phosphin-hydrochloride; Applichem, Germany) at 1:9 ratio. Subsequently, the sample was deproteinized using 1M $HClO_4$ (at 2:1 ratio) and applied to the HPLC/EC system.
Determination of thiol concentration. The samples were fed to the HPLC system (P580A; Dionex, Germany) coupled to an electrochemical detector (CoulArray 5600; ESA, USA). The analysis was performed in Termo Hypersil BDS C18 column (250 x 4.6 x 5µm) (Germany) in isocratic conditions, using the mobile phase of 0.15 M phosphate buffer, pH 2.9, supplemented with 12.5-17% acetonitrile for estimation of Hcy and Met and 0.15 M phosphate buffer (Accinni et al., 2000).
The system was controlled and the data were collected and processed using Chromeleon software (Dionex, Germany).

3.3.3 Analysis of Arg and ADMA concentrations

Preparation of samples and derivatization. Plasma and the standard, containing solution of Arg and ADMA (Sigma, USA) were diluted with water at the ratio of 1.5:1.0 and, then, they were deproteinised using 8M $HCLO_4$ at the ratio of 5:1. Directly before HPLC analysis the samples were subjected to derivatization in a solution containing 10 mg OPA per 100 µl methanol supplemented with 900 µl 0.4 M borate buffer (pH 8.5) and 5µl 2-mercaptoethanol at the ratio of 1:1 (Pi et al., 2000).
Analysis of Arg and ADMA. The samples were fed to the HPLC system (P580A; Dionex, Germany) coupled to a fluorescence detector (RF2000; Dionex, Germany). The analysis was performed in a Termo Hypersil BDS C18 column (250 x 4.6 x 5µm) (Germany) in an isocratic conditions using 0.1 M phosphate buffer, pH 6.75 with 25 % methanol as the mobile phase. Arg and its methylated metabolites were measured fluorimetrically at excitation and emission wavelengths of 340 nm and 455nm, respectively.
The system was controlled and the data were collected and processed using Chromeleon software (Dionex, Germany).

3.3.4 Results

The study indicated that in patients with the diagnosed PD (Table 9) the augmented export of Hcy to plasma, ($p<0.001$ as compared to the controls), was accompanied by increased

levels of circulating ADMA in analyzed neurodegenerative disease ($p<0.001$ as compared to the controls). In parallel, in the patients lower levels were observed of both Met ($p<0.01$ as compared to the controls), and Arg ($p<0.05$ as compared to the controls) expressed also by the lowered Met/Hcy and Arg/ADMA ratio ($p<0.001$ as compared to the controls).

Parameter	Controls (22-76 years)	Patients with PD (41-86 years)
Hcy	13.0 ± 4.3	20.1 ± 14.1***
Met	24.0 ± 6.9	18.7 ± 8.6**
Met/Hcy	2.1 ± 0.9	1.2 ± 0.8***
Arg	79.7 ± 24.4	68.4 ± 21.9*
ADMA	2.0 ± 1.0	3.8 ± 1.9***
Arg/ADMA	55.3 ± 40.5	25.8 ± 20.9***

Table 9. Homocysteine (μM), methionine (μM), asymmetric dimethylarginine (μM) and arginine (μM) concentrations in the patients with PD and in control group. Results are expressed as a means ± SD. The nonparametric test of Mann-Whitney was used. Differences significant at *$p<0.05$, **$p<0.01$, ***$p<0.001$, as compared to the controls.

Moreover, in the patients with PD (Fig. 4) development of the degenerative disease resulted in increased levels the risk factor for vascular diseases (Hcy), particularly pronounced in IVth stage of PD development ($p<0.05$ between Ist and IVth stage and between IInd and IVth stage of PD evolution). On the other hand, the Hcy remethylation product demonstrated a decreasing tendency only in the stage III of the disease, as compared to stage I of PD. In parallel, levels of the other analyzed risk factor of vascular diseases (ADMA) manifested higher correlation with concentration of its precursor. In parallel to the development of PD from stage I to stage IV of the disease evolution augmented levels of ADMA were accompanied by a decrease in the level of Arg (as compared to the Ist stage of PD). Also at the IInd stage of the degenerative disease evolution the highest levels of Met and ADMA and practically unaltered levels of Arg were accompanied by the lowest value of Arg/ADMA ratio ($p<0.01$ between stages I and II of PD evolution). In the IVth stage of PD development, however, both Met/Hcy ratio and Arg/ADMA ratio behaved in a similar manner demonstrating practically the lowest level ($p<0.05$, as compared to the Ist stage of the disease development).

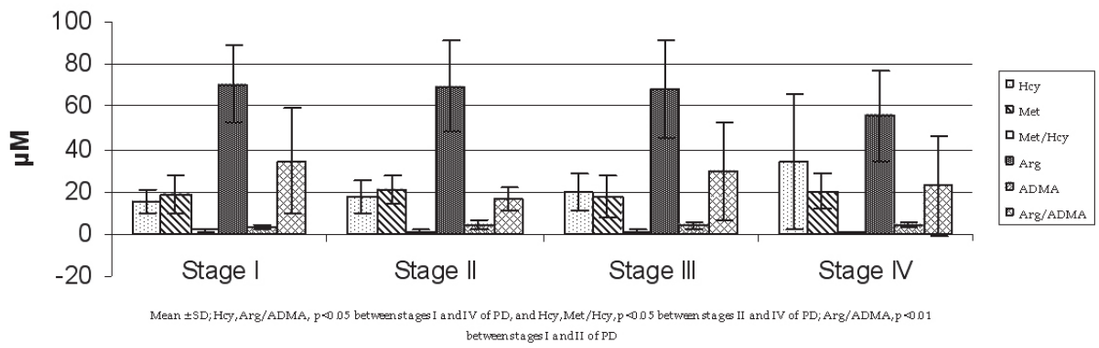


Fig. 4. Hcy, Met, Arg and ADMA concentrations as related to the stage of the PD according to the scale of Hoehn and Yahr.

Pharmacotherapy with L-dopa preparations was demonstrated also to increase levels of both factors of vascular disease risk (Table 10), Hcy ($p<0.001$ as compared to the controls and $p<0.05$ as compared to patients not treated with L-dopa) and ADMA ($p<0.001$ as compared to the controls), although levels of ADMA increased also as a result of development of the degenerative disease ($p<0.01$ as compared to the controls). In parallel, in patients treated with L-dopa preparations levels of Met decreased ($p<0.001$ as compared to the controls) and so did concentrations of Arg ($p<0.05$ as compared to the controls and to L-dopa untreated patients), Met/Hcy ratios ($p<0.001$ as compared to the controls and $p<0.05$ as compared to patients not treated with L-dopa) and Arg/ADMA ratios ($p<0.001$, only as compared to the controls).

Parameter	Controls (22-76 years)	Patients with PD L-dopa (-) (41-78 years)	Patients with PD L-dopa (+) (46-86 years)
Hcy	13.0 ± 4.3	15.1 ± 5.1	22.0 ± 15.9$^{***(*)}$
Met	24.0 ± 6.9	21.0 ± 7.8	17.8 ± 8.8***
Met/Hcy	2.1 ± 0.9	1.6 ± 1.0*	1.0 ± 0.6$^{***(*)}$
Arg	79.7 ± 24.4	77.5 ± 19.7	64.4 ± 22.0$^{*(*)}$
ADMA	2.0 ± 1.0	3.3 ± 1.1**	4.1 ± 2.1***
Arg/ADMA	55.3 ± 40.5	22.8 ± 7.8***	26.7 ± 23.6***

Table 10. Homocysteine (μM), methionine (μM), asymmetric dimethylarginine (μM) and arginine (μM) concentrations as related to pharmacotherapy with L-dopa (+) in the patients with PD. The nonparametric test of Mann-Whitney was used. Results are expressed as means ± SD. Differences significant at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, as compared to the controls. Differences significant at $^{(*)}p<0.05$, as compared to patients not treated with L-dopa (-).

Present study indicated that ADMA may be involved in pathogenesis of PD. In development of PD the principal role is thought to be played by peroxinitrates (Padovan-Neto et al., 2009). This seems consistent with the demonstrated in present study ADMA level not elevated till the IInd stage of the disease development and the lower level of Arg, particularly accentuated in the IVth stage of PD evolution. Thus, a probability exists for involvement of reactive NO derivatives in induction of toxic damage to *substantia nigra* in PD.

In present study we have observed particularly in PD patients an evident decrease in Arg/ADMA ratio. The lowered ratio in blood is thought (Matsuoka et al., 1997) to be linked to development of hypercholesterolemia, congestive heart failure, arterial occlusive disease, heart failure and hypertension.

The levels of ADMA in PD have probably been affected also by pharmacotherapy with L-dopa. Both in the studies of Qureshi et al. (1995), and in present study decreased levels of Arg have been shown in patients treated with the drug. In the study of Qureshi et al. (1995) pharmacotherapy with L-dopa has been shown to generate nitrites, agents of neurotoxic activity, but in present studies seem that ADMA has not been shown to participate in their generation. Increased NO levels in PD have seemed to result rather from elevated activity of the glutaminergic system and altered neuronal metabolism.

It seems that ADMA may be regarded to represent a risk factor for PD and may be involved in pathogenesis of this neurodegenerative disease. Present results indicate also that

developing neurodegenerative diseases are accompanied by disturbed metabolism of Hcy and ADMA and administration of L-arginine, in line with vitamins B6, B12 and folates, to PD patients may offer a modern therapy in this neurodegenerative disease.

4. Polymorphisms of *MTHFR, MTR, MTHFD1* and the level of biothiols in Parkinson's disease

The enzyme MTHFR plays a key role in regulating of Hcy metabolism. The study of Yasui et al. (2000) indicated that the TT *MTHFR* C677T genotype might also be linked to pathogenesis of PD, particularly when the level of folates is low. However, Fong et al. (2011) indicated on synergistic effects of polymorphisms in the folate metabolic pathway genes in PD not only C677T *MTHFR*, but also A2756G of *MTR* and A1049G, C1783T of *MTRR* (methionine synthase reductase). Yuan et al. (2009) showed on synergism between Hcy elevation after L-dopa administration in PD patients and *MTHFR,* CT and TT (C677T) genotypes. The study of Dorszewska et al. (2007) indicated that the genotypes TT (C677T), CC (A1298C) and AA (G1793A) of *MTHFR* have been the least frequent in patients with neurodegenerative disorders and their incidence has been slightly increased in the degenerative diseases (e.g. PD). In study of Dorszewska et al. (2007) also indicated that in PD patients Hcy has reached higher levels only in patients with CT genotype of *MTHFR* C677T. It seems that, particularly in persons with CT genotype of the C677T polymorphism of *MTHFR*, processes of Hcy transsulfuration to Cys become disturbed. Moreover, in PD the most pronounced alterations in Hcy levels in cases of A1298C polymorphism of *MTHFR* have been manifested in both genotypes AA and AC, even if this has not been expressed by the increased in parallel levels of oxidized guanine in DNA.

The enzyme of MTR is responsible for transfer of methyl groups from methyltetrahyrofolate to Hcy with involvement of methylcobalamin as the cofactor. The AG genetype of the A2756G polymorphism of *MTR* is probably linked to augmented levels of Hcy (Dorszewska et al., 2007). The increase of Hcy concentrations most probably results from lowered activity of MTR, induced by excessive oxidation of cobalamin (McCaddon et al. 2002) due to a more pronounced oxidative stress in degenerative syndromes. In parallel, the studies of Matsuo et al. (2001) indicate that *MTR* AG leads also to hypomethylation of DNA and to inactivation of several genes (low levels of SAM).

It seems also that the polymorphism G1958A of the gene coding for MTHFD1 enzyme may be involved in pathogenesis of PD, and heterozygote as well as homozygote (GA, AA) are thought to be responsible for increased levels of Hcy (Dorszewska et al., 2007). MTHFD1 represents another folate-dependent enzyme, which catalyzes transformation of tetrahydrofolate to 10-formyl, 5,10-methenyl and 5,10-methylene derivatives. 10-Formyltetrahydrofolate and 5,10-methylenetetrahydrofolate are regarded to serve as donors of methyl groups in DNA biosynthesis. The study of Dorszewska et al. (2007) also indicated that significant differences of the levels of Cys/Hcy, *MTHFD1* GA (G1958) were between AD and PD groups. The results indicate that only polymorphisms of folate-dependent enzyme *MTHFD1* have pointed to significant differences in intensity of turnover of circulating thiols between both neurodegenerative diseases, AD and PD.

Some studies confirmed that the C677T *MTHFR* polymorphism should consider as a genetic risk factor in patients who are going to take L-dopa preparations and folates and vitamins B (6, 12) supplementation may be given to the treated PD patients.

5. Influence of L-dopa treatment duration on the level of oxidative damage to DNA and thiols compounds concentration in patients with Parkinson's disease

The discussion about value of the L-dopa treatment in PD concerning on: toxicity, biochemical effects, clinical motor complications, especially after long-term its administrations (Belcastro et al., 2010; Muller, 2009a). Long-term treatment with L-dopa in PD patients may be promotes Hcy levels increase. Moreover, only PD patients with hyper-Hcy (Hcy above 15 μM) may have disturbed metabolism Hcy to Cys. As showed, hyper-Hcy in PD patients has been correlated with duration of disease and L-dopa dose.

5.1 Patients (see point 2.1.1)
5.2 Analysis of Hcy and Met (see point 3.3.2), and Cys (see point 3.2.2) concentrations, and 8-oxo2dG level (see point 2.1.2)
5.3 Results

During the initial five years and within the following 10 years of treatment with L-dopa (Table 11), the levels of 8-oxo2dG were augmented ($p<0.05$, as compared to the controls). Similarly to 8-oxo2dG, the levels of Hcy were highest after the initial five years of L-dopa administration ($p<0.05$, as compared to the controls). Subsequent treatment for another five to ten years resulted in the elevated levels of Hcy ($p<0.01$, as compared to the controls) which were even more significant if the treatment was extended over ten years ($p<0.001$, as compared to the controls). Moreover, the initial five years of L-dopa treatment were accompanied by relatively low levels of Met ($p<0.05$, as compared to the controls) and a slight increase in concentration of Cys. After ten years of treatment, similar levels of Hcy and Met were detected (Met, $p<0.01$), as compared to the controls, and Cys, ($p<0.05$), as compared to the group treated for five to ten years.

Parameter	Controls (22-76 years)	Patients with PD		
		up to 5 year treatment (34-78 years)	5-10 year treatment (46-81 years)	over 10 year treatment (46-81 years)
8-oxo2dG	13.7 ± 7.6	21.5 ± 15.1*	17.5 ± 11.1	27.8 ± 23.0*
Hcy	12.6 ± 4.3	28.5 ± 33.6*	19.7 ± 9.0**	18.3 ± 6.9***
Met	24.2 ± 6.7	19.2 ± 6.2*	20.4 ± 9.1	18.2 ± 8.2**
Met/Hcy	2.2 ± 0.9	1.1 ± 0.5**	1.2 ± 0.6**	1.1 ± 0.5***
Cys	220.7 ± 46.6	232.3 ± 52.5	267.9 ± 47.1**	238.2 ± 53.3(*)
Cys/Hcy	19.3 ± 6.7	12.8 ± 5.3*	15.4 ± 5.1	14.4 ± 5.8**

Table 11. Levels of oxidative DNA damage (8-oxo2dG/dG x 10^{-5}), and homocysteine (μM), methionine (μM) and cysteine (μM) concentrations as related to duration of L-dopa administration to patients with PD. Results are expressed as means ± SD. Differences significant at *$p<0.05$, **$p<0.01$, ***$p<0.001$, as compared to the controls. Differences significant at (*)$p<0.05$ between patients treated with L-dopa for 5-10 year and those treated for over 10 year.

As shown by the literature (Spencer et al., 1995) and by our studies, the elevated level of oxidized guanine in DNA (8-oxo2dG) in PD reflects also pharmacotherapy with L-dopa preparations. In present study, levels of 8-oxo2dG in the patients treated with L-dopa

preparations have reflected duration of administration of the drug. Patients have seemed most exposed to oxidative stress, resulting from L-dopa administration, during the first 5 years of treatment with the preparation and following long-term (over 10 years) its administration. According to Spencer et al. (1995), the augmented oxidative stress in patients treated with L-dopa might have resulted from lowered levels of antioxidants (GSH), disturbed mitochondrial transport and from excessive oxidation of dopamine.
Reports of the literature (Miller et al., 2003) and present results indicate that plasma Hcy levels in PD have been affected also by pharmacotherapy with L-dopa. In present study levels of the sulfuric amino acid were also affected by duration of the pharmacotherapy. The most exposed to neurotoxic effects of Hcy have seemed to be the patients during the first 5 years L-dopa treatment while its continued administration has resulted in stably elevated Hcy level, it seems that all time are disturbed metabolism of Hcy to Met and Cys.
Treatment with L-dopa preparations seems to be a potential risk factor for vascular diseases in PD patients. According to Lamberti et al. (2005), administration of vitamin B12 and of folates decreases plasma level of Hcy particularly in patients with PD during treatment with L-dopa preparations and in this way prevents against intensification of vascular lesions and dementia in the patients.

6. Conclusion

In conclusion, L-dopa metabolism after administration in PD patients is an important component for Hcy elevation and for increase toxicity in peripheral blood lymphocytes. Therapy of L-dopa leads to increases of the level factors inducing in oxidative stress and apoptosis as well as changes concentrations of risk factors of vascular diseases such as: Hcy, Cys and ADMA especially after long-term therapy. Analysis of the level of 8-oxo2dG, Hcy, Cys and ADMA may be a new biomarkers of severity and progression of PD.
It seems that for elevated levels of biothiols in PD, is not only important genotype *MTHFR,* TT (C677T) but also CC (A1298C), AA (G1793A), and *MTHFD1,* AA (G1958A) and *MTR,* GG (A2756G), which have a tendency for increased frequency in PD patients. In PD, there are more significant differences of the levels of biothiols in patients with one of genotype: Hcy [*MTHFR*: CT (C677T) and GG (G1793A); *MTR*, AG (A2756G)], Met [*MTR*, AA (A2756G)], Cys [*MTR*, AG (A2756G)], and Met/Hcy [*MTHFR*: CC, CT (C677T) and AA (A1298C), and GG (G1793A); *MTHFD1* AA (G1958A); *MTR* AA (A2756G)]. Moreover only polymorphisms of folate-dependent enzyme *MTHFD1* have pointed to significant differences in intensity of turnover of circulating biothiols between both neurodegenerative diseases: AD, and PD, which differ in the localization of neurotoxic lesions in the CNS.
Therefore in PD, monitoring of thiols compound levels in particular Hcy, is recommended. In the patients with PD administration of vitamins B6, B12, folates may cause a decrease in Hcy level, due to increased efficiency of remethylation and transsulfuration processes.

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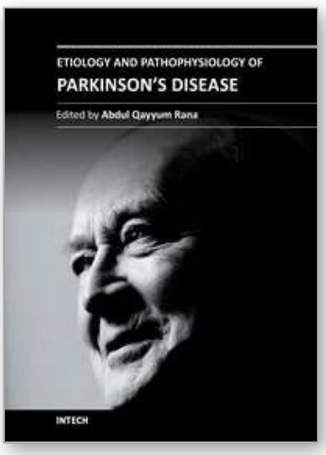
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This book about Parkinsonâ€™s disease provides a detailed account of etiology and pathophysiology of Parkinsonâ€™s disease, a complicated neurological condition. Environmental and genetic factors involved in the causation of Parkinsonâ€™s disease have been discussed in detail. This book can be used by basic scientists as well as researchers. Neuroscience fellows and life science readers can also obtain sufficient information. Beside genetic factors, other pathophysiological aspects of Parkinsonâ€™s disease have been discussed in detail. Up to date information about the changes in various neurotransmitters, inflammatory responses, oxidative pathways and biomarkers has been described at length. Each section has been written by one or more faculty members of well known academic institutions. Thus, this book brings forth both clinical and basic science aspects of Parkinsonâ€™s disease.

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