Trakia Journal of Sciences, Vol. 10, Suppl. 1, 46-51, 2012

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

EFFECT OF MADOPAR THERAPY ON TWO OXIDATIVE STRESS BOIMARKERS IN PATIENTS WITH PARKINSON DISEASE – A COMPARATIVE STUDY

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ABSTRACT

Parkinson's disease (PD) is one of the major progressive neurological disorders for which no preventative or long-term effective treatment strategies are available. This is a slow, progressive disease and is characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra (SN) and intraneuronal cytoplasmic inclusions called Lewy bodies. The causes for degeneration of dopamine neurons are not well understood.

Numerous hypotheses have been proposed to explain the neurodegenerative mechanisms that occur in PD and some of them show that oxidative stress plays a considerable role. Imbalance between levels of ROS resulting in the body and the capacity of antioxidant defense mechanisms provokes oxidative stress (OS).

The aim of this study was to evaluate and compare the effect of Madopar therapy in PD patients on the both oxidative stress biomarkers: 1) the level of ascorbate free radicals as a real-time biomarker and 2) the level of malondialdehyde (MDA) as reactive end products of the lipid peroxidation. Our data demonstrated that redox balance in the sera of PD patients without therapy compared to those of PD patients treated by Madopar and healthy volunteers was considerably disturbed. Moreover, application of the drug improved to a great extent the oxidative status of the PD patients.

Key words: Ascorbate radicals; Oxidative stress; MDA, Parkinson disease.

INTRODUCTION

The word Parkinsonism refers not to a particular disease but to a commonly recognized condition marked by characteristics set of symptoms (1). Chief among these are trembling of the limbs, muscular stiffness, and slowness of bodily movement. This complex of symptoms are called Parkinsonism reflects the dysfunction of particular region of the brain–in fact, of a particular system of nerve cells in a center or nucleus known as the substantia nigra (SN). There is a lot of data that the pigment from SN is related in some way to these nerve cells which produce and store a specific chemicals substance called dopamine. Dopamine produced in these nerve cells act as a chemical messenger transmitting signals to the nerve cells of the striatum (2). When the substantia nigra cells are injured or for same reason cannot produce or store dopamine, there results a deficiency of dopamine in striatum. If the deficiency is sufficiently severe, symptoms of Parkinsonism begin to appear. Some neuroscientists have defined Parkinsonism in chemical terms as a state of brain dopamine depletion (1).

The brain is only 2-3% of the total body mass, but it consumes 20% of basal oxygen (3, 4). Cells in the brain are particularly susceptible to oxidative damage due to the high concentrations of polyunsaturated fatty acids in their membranes and relatively low activity of endogenous antioxidant enzymes (3). Aging is associated with an increased oxidative stress and accumulation of oxidative damage to

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biomolecules which gradually weakens cognition (3, 5). More oxidized proteins, including carbonyls and nitro-protein adducts are found in the brains of elderly (3).

The cause of Parkinson's disease is not known, but many experimental studies have shown that oxidative stress and reactive oxygen species (ROS) play a key role and that ROS are involved in many complications of that disease (6). Among the many biological targets of oxidative stress, lipids are the most vulnerable class of biomolecules. Lipid peroxidation (LPO) is often a late event, accompanying rather than causing final cell death (7), and does appear to make a significant contribution to the development of Parkinson disease. LPO gives rise to a number of secondary products. They are mainly aldehydes, with the ability to exacerbate oxidative damage (6). In view of their longevity and high reactivity these products can act inside and outside the cells, interacting with biomolecules such as nucleic acids and proteins, often irreversibly damaging the delicate mechanisms involved in cell functionality. MDA is the principal and most studied end product of the LPO process. An increased concentration of these products is the evidence for most frequently quoted for the involvement of free radicals in human disease (8, 9, 10).

The ascorbate anion, AH^-, is an endogenous water-soluble antioxidant presenting in biological systems (11). After one-electron oxidation ascorbate anion produces ascorbate free radical ($Asc^{\cdot}$), which is easily detectable by EPR (12) in aqueous solution at room-temperature.

In a number of studies was documented that concentration of ascorbate radical was a reliable, real-time and quantitative marker of free radical generation (11) and that the intensity of the EPR spectrum of ascorbate free radical could be used as an indicator of oxidative stress *in vitro* and *in vivo* (11, 13, 14).

In the present study for the first time we have evaluated the effect of the Madopar therapy on the oxidative status of PD patients by following up and comparing the changes of the level of ascorbate radicals as a real-time oxidative stress biomarker and the level of MDA as reactive end LPO products, measured in blood samples of PD patients.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased by Sigma Chemical Co, St. Louis, USA and were analytical grade.

Methods

Spectrophotometry

All spectrophotometric measurments were performed on a ThermoScientific spectrophotometer, at OD 532nm.

Electron paramagnetic resonance (EPR) studies

All EPR measurements were performed at room temperature on a X-band EMX^{micro}, spectrometer Bruker, Germany, equipped with standard Resonator. All EPR experiments were carried out in triplicate and repeated thrice. Spectral processing was performed using Bruker WIN-EPR and Simphonia software.

Patients and biological material

Blood samples obtained from 14 patients with Parkinson's disease, treating with Madopar (7 males and 7 females), 10 PD patients without therapy (5 males and 5 females) all of them are patients of Neurological clinic of University Hospital, Stara Zagora, Bulgaria and 14 healthy volunteers (7 males and 7 females) who had no family history of Parkinson's disease, were used as controls.

To eliminate the factors that might affect parameters of oxidative stress, we excluded from Parkinson's patients and healthy controls all smoking and alcohol-drinking subjects, as well as individuals suffering from acute or chronic diseases.

Informed consent was obtained from all participants in the study according to the ethical guidelines of the Helsinki Declaration.

Laboratory analysis

Fasting samples of venous blood were collected in the morning between 8.00 and 10.00 h. Blood samples for measuring the level of MDA reactive products were collected in tubes containing 10% EDTA -ethylene diaminetetraacetic acid, centrifuged at 3,000 rpm for 15 min, and plasma samples carefully separated.

Sera for evaluation the level of ascorbate radical were obtained by centrifugation of the blood samples at 3,000 rpm for 5 min, and carefully separated.

Determination of the lipid peroxidation products

The method of thiobarbituric acid (TBA), which measures MDA reactive products, was used the method described by Plaser and Cushman, 1966 (15) with modifications by Gadjeva et. al. 2005, (16). In brief, 1 ml plasma, 1 ml physiological solution, and 1 ml 25% trichloroacetic acid were mixed and centrifuged at 7,000 rpm for 20 min. 2 ml protein-free supernatant was mixed with 0.5 ml 1% TBA and heated at 95ºC for 1 h. After cooling, the intensity of pink colour of the end fraction product was determined at 532 nm. The MDA concentration was calculated according to the following formula:

$$1\mu mol/l\ MDA = \frac{OD_{532} \times 1.75}{0.156}$$

OD532 - optic density in λ=532 nm and extinction= 1.56 x 105 M^{-1} cm^{-1}

EPR ex vivo evaluation the level of ascorbate radicals in the sera of PD patients and healthy volunteers

The level of ascorbate radical was measured as described by Bailey D. et al. 2004 and modified by A. Zheleva et. al. 2012. In brief, the sera from patients and volunteers were prepared in DMSO in a ratio of 1:3. After centrifugation the supernatants were collected and immediately transferred into a quartz tubes and placed in EPR cavity. EPR settings were as follows: 3505.00 G center field, 20.00 mW microwave power, 1.00 G modulation amplitude, 15 G sweep width, a receiver gain $1x10^5$, 40.96 ms time constant, 60.42s sweep time, 10 scans per sample.

Statistical analysis

Statistical analysis was performed with Statistica 7, StaSoft, Inc. and results were expressed as means ± standard error (SE). Statistical significance was determined by the Student's t-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCASSION

In all studied sera a typical EPR doublet signal of an ascorbate free radical was recorded **(Fig. 1 A, B)** with values of g factor and a_H splitting constant close to those previously reported (11). As is seen on **Fig. 2** the level of ascorbate radicals registered in sera of PD patients without therapy compared to those of the PD patient treated with Madopar (mean 3748125± 132386.2, vs, mean 2090176 ± 197719.5, p=0.00) and to the controls (mean 3748125 ±132386.2, vs mean 860962 ± 17794.7, p= 0.00) were statistically significant higher. Almost the same dependence was established for the levels of MDA products. As is seen on Fig. 3 MDA levels in PD patients without therapy compared to those treated by Madopar (mean 3.26 μM vs mean 2.62 μM ± 0.1, p=0.00) and to the controls (mean 3.26μM ±0.08, vs mean 2.39μM ± 0.04, p=0.00) were statistical significantly higher. Increased level of MDA products registered in both patients groups confirm that LPO process takes place in PD. Moreover, as was demonstrated, Madopar considerably improved the oxidative status of the treated patients.

It is well known that generation of reactive oxygen species (ROS) are involved in initiation and progression of the LPO processes in biological systems (14). Another confirmation for ROS generation in the present study were statistically higher levels of ascorbate radical ($Asc^{\cdot}$) registered in patients without therapy comparing to the healthy controls and Madopar treated patients (**Fig. 2**).

Ascorbate is situated thermodynamically at the bottom of the oxidizing radicals order which means that all oxidizing species with higher redox potentials ($^{\cdot}OH$, $^{\cdot}ROO$, $^{\cdot}LOO$, $^{\cdot}TO$, $ONOO^{-}$ and others) could be repaired by it, leading to generation of $Asc^{\cdot}$ (16)**.** Results from our EPR spectroscopy study demonstrate that not only oxidizing free radicals causing LPO might be involved in PD oxidative damages but other oxidizing radicals also might participate in PD oxidative stress. These radicals are probably provoke additional generation of $Asc^{\cdot}$ that can not be overcame by application of Madopar therapy and might act *in vivo* as an prooxidant. Moreover, catalytically active transition metals such are iron or copper also might be important generators of $Asc^{\cdot}$ in biological systems (18). This assumption is supported by our finding that ratio between the levels of MDA measured in Madopar treated patients and controls is lower comparing to that ratio between the levels of $Asc^{\cdot}$ in drug treated patients and controls. Obviously, Madopar is more effective as antioxidant only in respect of LPO process developed in PD patients.

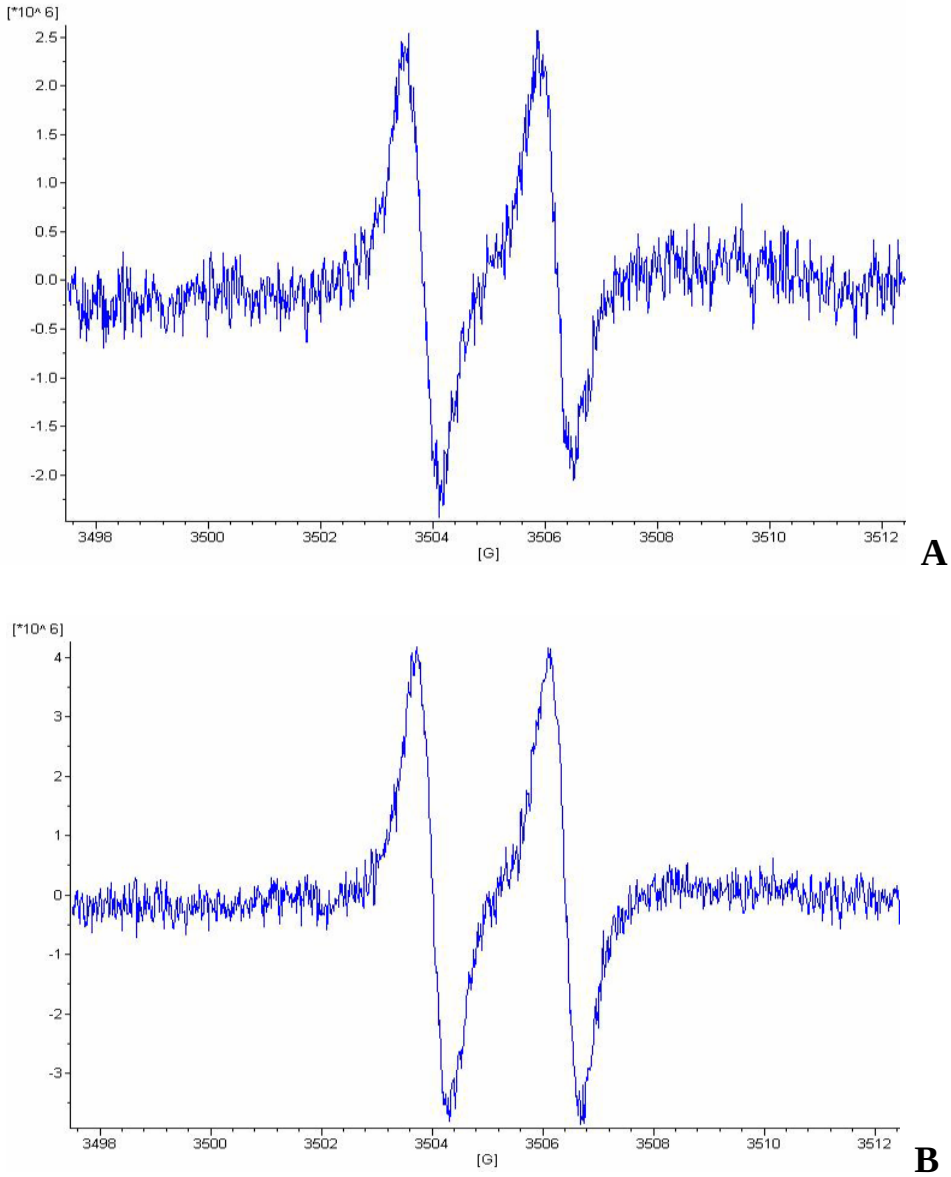

Fig. 1. EPR spectra of the ascorbate radicals registered in sera of PD patients with therapy – Madopar (A) and in sera of PD patients without therapy (B).

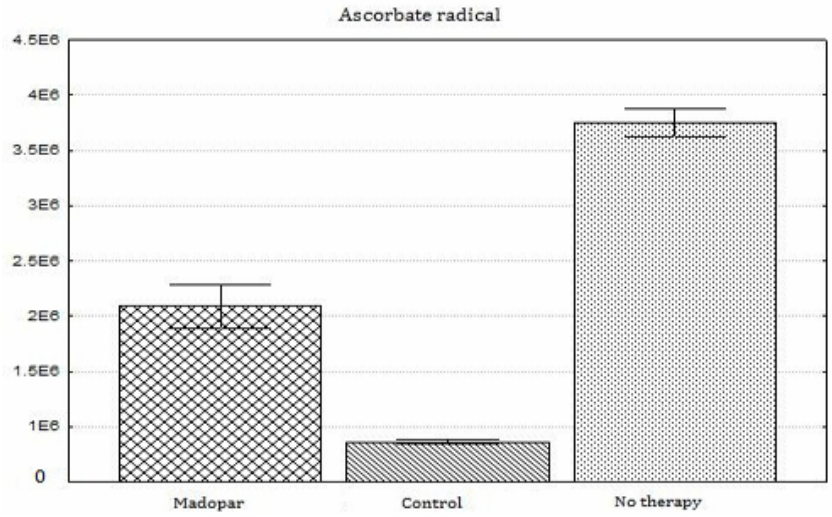

Fig. 2. The levels of ascorbate radicals measured by the intensity of the EPR signal (arb. units) in the sera of PD patients with therapy –Madopar and in PD patients without therapy and controls.

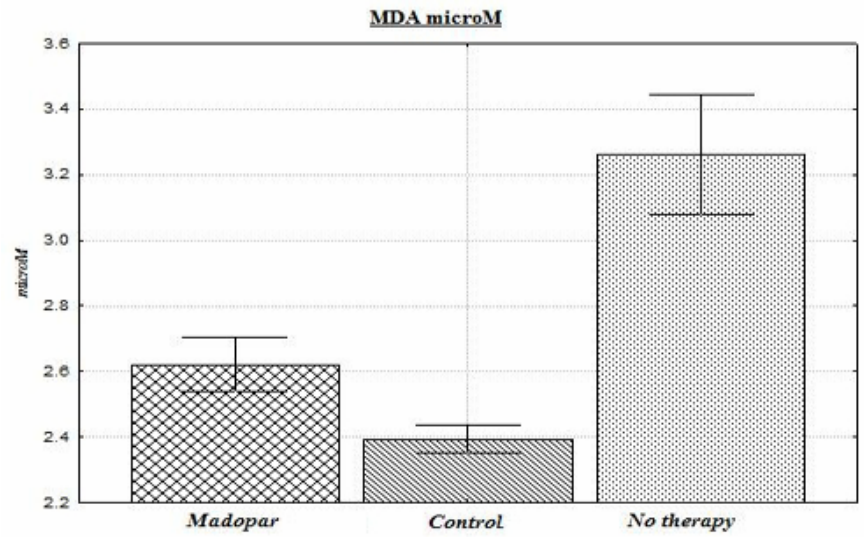

Fig. 3. The levels of MDA in plasma of patients with therapy, patients without therapy and controls.

CONCLUSIONS

Our data demonstrated that redox balance in the sera of PD patients without therapy compared to those of PD patients with Madopar therapy and healthy volunteers is considerably disturbed and application of Madopar improves the oxidative status of the treated PD patient in respect of LPO process. In this connection we consider that combined therapy in PD patients treated with Madopar and other suitable antioxidants which cross the blood brain barrier will reduce the level of free radicals and that is the subject of our future research.

ACKNOWLEDGMENTS

This work was financed by the European Social Fund through Operative programme "Development of human resources" 2007-2013, Contract №BG051PO001-3.3.06-0006 "Support for development of PhD students, post doctorals, post-graduate students and young scientists".

REFERENCES

1. Duvoisin, R.C. Parkinson's disease "A guide for Patient and family", Raven Press, N.Y., 1985
2. Halliwell B., Role of free radicals in the neurodegenerative diseases. *Drugs Aging* 18:685–716, 2001
3. Mariani, E., Polidori, M. C., Cherubini, A., Mecocci, P., Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. *J of Chromatography* B: Analytical Technologies in the Biomedical & Life Sciences, 27: 65-75, 2005.
4. Moreira, P. I., Siedlak, S. L., Aliev, G., Zhu, X., Cash, A. D., Smith, M. A., Perry, G., Oxidative stress mechanisms and potential therapeutics in Alzheimer disease. *J of Neural Transmission*, 112: 921-32, 2005
5. Cardoso, S. M., Moreira, P. I., Agostinho, P., Pereira, C., Oliveira, C. R., Neurodegenerative pathways in Parkinson's disease: therapeutic strategies. *Current Drug Targets* - CNS & Neurological Disorders, 4: 405-19. 2005.
6. Halliwell B, Alliwell B, Grootveld H., The measurement of free radical reactions in humans. FEBS Lett, 213: 9–14, 1987.
7. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 2nd ed. Oxford: Clarendon Press., 1989.
8. Halliwell B. and Chirico S., Lipid peroxidation: its mechanism, measurement and significance. *American Society for Clinical Nutrition*, 57(suppl):7 15S-25S, 1993.
9. Daniele Del Rio, Amanda J. Stewart, Nicoletta Pellegrini, A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism & Cardiovascular Diseases*, 15, 316-328, 2005.
10. Pryor WA, Stanley JP., A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymatic production of prostaglandin endoperoxides during autoxidation (Letter). *J Org Chem*;40:3615-3617, 1975.
11. Buettner, G.R.; Ascorbate radical: A Realtime Indicator of Oxidative Flux.

SFRBM November, Workshop: Rigorous Detection and Identification of Free Radicals in Biology and Medicine, 2005.

12. Rota C, Fann YC, Mason RP. Phenoxyl free radical formation during the oxidation of
1. the fluorescent dye 2',7'-dichlorofluorescein by horseradish peroxidase. Possible consequences
2. for oxidative stress measurements. *J Biol Chem*, 274:28161-8, 1999.
13. Buettner, G.R.and Jurkiewlcz, B. A.: Ascorbate free radical as a marker of oxidative stress:
3. An EPR study, *Free Radical Biology & Medicine*, 14:49-55, 1993.
14. Sharma, G.R., Buettner, K.T., Spencer, K.T., Kerber, R.E., Ascorbyl free radical as a real-time marker of free radical generation in briefly ischemic and reperfused hearts. An electron paramagnetic resonance study. *Circ. Res*. 74: 650-658, 1994
15. Plaser ZA, Cushman LL, Jonson BC, Estimation of product of lipid peroxidation (Malonyl Dialdehyde) in biochemical systems. *Anal Biochem* 16:359–364, 1966.
16. Gadjeva V, D. Kuchukova, R. Georgieva (2005). Influence of polychemotherapy on the antioxidant levels and lipid peroxidation in patients with lymphoproliferative diseases. Comparative Clinical Pathology 14, (1)13-18.
17. Antoaneta Zheleva, Yanka Karamalakova, Galina Nikolova, Veselina Gadjeva. New Possibilities For Treatment Of Amatoxin Intoxications: An EPR Spectroscopy Study On Free Radical Formation Caused By The Mushroom Toxin Alpha Amanitin. Pakis. J. Pharm. Sci. (In Press).
18. Spasojevic, Kojic D., Mojovic, I., Blagoevic, M., Worland, D., Grubor-Lajsic, M.R., and Spasic, M.B. Potential role of hydrogen peroxide and melanin in the cold hardiness of Ostrinia nubilalis. *Eur.J Entomol*, 106: 451-454, 2009.