ERYTHROCYTE SUSCEPTIBILITY TO LIPID PEROXIDATION AND ANTIOXIDANT SYSTEM IN PARKINSON'S DISEASE PATIENTS

A.Kılınç*** / A.S. Yalçın** / D. Yalçın**** / Y. Taga ** / K. Emerk *

* *Professor, Department of Biochemistry, Faculty of Medicine, Marmara University, İstanbul, Turkey.*
** *Associate Professor, Department of Biochemistry, Faculty of Medicine, Marmara University, İstanbul, Turkey.*
*** *Research Assistant, Department of Biochemistry, Faculty of Medicine, Marmara University, İstanbul, Turkey.*
**** *Research Assistant, Department of Neurology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Turkey.*

SUMMARY

It has been suggested that among the factors that lead to neuron loss in substantia nigra in Parkinson's disease are stimulation of lipid peroxidation and deficiency of glutathione and glutathione peroxidase. In this study, the effect of neurodegenerative changes on plasma and erythrocytes were investigated in Parkinson's disease. The results were compared with age-matched controls. Increase in plasma lipid peroxide levels and erythrocyte susceptibility to lipid peroxidation were observed in patients with Parkinson's disease. Erythrocyte catalase activities were not changed whereas glutathione levels and glutathione peroxidase activities were decreased.

Our results suggest that erythrocyte membrane integrity is impaired in Parkinson's disease. We are carrying out more detailed studies to determine whether this is related to neurodegenerative changes observed in Parkinson's disease and whether parkinsonians will benefit from antioxidant treatment.

Key words: Antioxidant system, Plasma, Erythrocyte, Lipid peroxidation, Parkinson's disease

INTRODUCTION

During the course of normal cellular metabolism, the participation of molecular oxygen in processes involving electron transport and utilization of oxygen by a variety of chemical reactions results in the formation of superoxide radicals, and hydrogen peroxide. These in turn may interact to produce singlet oxygen and hydroxyl radicals. All of these reactive oxygen species are capable of disrupting a variety of biomolecules, including proteins, nucleic acids and especially polyunsaturated fatty acids of cell membranes (1).

Each cell is protected by an extremely effective network of antioxidant defence system (2). This system includes enzyme scavengers such as superoxide dismutase, catalase, and glutathione peroxidase. There are also endogenous free radical scavengers. Among these, reduced glutathione plays an important role in the protection of cell membranes from damage due to peroxides and free radicals. Tocopherols may also act as free radical scavengers and singlet oxygen quenching agents. Intracellular formation of peroxides are reduced by glutathione peroxidase, with selenium as a metallic cofactor and glutathione as a cosubstrate.

On the other hand, Parkinson's disease is characterized by a severe disturbance of the extrapyramidal motor system. Although the biochemical basis for the extrapyramidal symptoms of Parkinson's disease, namely the brain nigrostriatal dopamine deficiency has been established, the factors leading to the degeneration of the nigral neurons are not known (3). One suggestion is that neuronal degeneration is a consequence of excessive lipid peroxidation provoked by free radicals and reactive oxygen species (4). It has been reported that protective mechanisms, i.e. nigral glutathione, catalase and glutathione peroxidase are reduced in Parkinson's disease (5 - 7). However, there is still much controversy about the increase in lipid peroxidation and its role in nigrostriatal cell death (8, 9).

Since these changes would not be restricted to one organ or cell type, we have decided to investigate these metabolic processes outside the central nervous system, namely in plasma and erythrocytes which are easily accessible.

MATERIALS AND METHODS

Heparinized blood was obtained from Parkinson's disease patients who applied to outpatient clinics of Department of Neurology, Istanbul Faculty of Medicine. All patients were already on L-DOPA treatment. Age-matched healthy individuals were used as controls.

Plasma lipid peroxide levels were determined by the

thiobarbituric acid test (10) and were expressed in terms of malondialdehyde (MDA). Glutathione transferase activities were determined according to Habig et al. (11). Erythrocyte susceptibility to lipid peroxidation was determined by MDA formation, with the "addition technique" of Stocks et al.(12) in which erythocyte suspensions were incubated with hydrogen peroxide for 2 hr at 37°C. Erythrocyte glutathione levels and catalase activities were determined according to Beutler (13, 14). Glutathione peroxidase activities were assayed by using t-butylhydroperoxide as substrate (15). Hemoglobin concentrations were determined using commercial kits (Wako-W.Germany).
The results from Parkinson's disease patients were compared with controls and the level of significance was assayed by Student's t-test. The results were also subjected to correlation and linear regression analysis.

RESULTS

As shown in Table I , there was a significant ($P < 0.001$) increase in both plasma lipid peroxide levels and erythrocyte susceptibility to lipid peroxidation in the Parkinson's disease patients. Median corpuscular fragility values in the two groups were not significantly different from each other.

Table I. Plasma lipid peroxide levels, erythrocyte susceptibility to lipid peroxidation and erythrocyte median corpuscular fragility (MCF) values in Parkinson's disease patients and healthy controls (mean ± S.E.M.)

	CONTROL (n=25)	PARKINSON'S DISEASE (n=20)
Plasma Lipid Peroxide (nmol MDA / ml plasma)	3.16 ± 0.14	5.72 ± 0.56 *
Erythrocyte Lipid Peroxidation (nmol MDA / g Hb)	474.4 ± 16.9	586.2 ± 24.0 *
Erythrocyte MCF (g/L)	4.28 ± 0.03	4.31 ± 0.06

* $P < 0.001$ when compared to controls

Table II lists erythrocyte glutathione levels as well as erythrocyte catalase, glutathione peroxidase and plasma glutathione transferase activities. In the Parkinson's disease patients there was a slight but significant ($P < 0.05$) reduction of glutathione levels. In addition, erythrocyte catalase activities were not changed, whereas glutathione peroxidase activities were significantly ($P < 0.001$) decreased in the Parkinson's disease patients. Plasma glutathione transferase activities were observed to be significantly increased ($P < 0.001$) in this group.

Correlation and linear regression analysis showed that there was no significant correlation between the parameters investigated.

Table II. Erythrocyte glutathione levels, erythrocyte catalase, glutathione peroxidase and plasma glutathione transferase activities in Parkinson's disease patients and healthy controls (mean ± S.E.M.)

	CONTROL (n=25)	PARKINSON'S DISEASE (n=20)
Erythrocyte glutathione (micromol/g Hb)	8.2 ± 0.2	7.5 ± 0.3 **
Erythrocyte catalase (IU/g Hb) x 1000	178.7 ± 6.5	181.2 ± 7.0
Erythrocyte GSH peroxidase (IU/g Hb)	28.6 ± 0.6	17.2 ± 0.7 *
Plasma GSH transferase (nmol product / minute/ml)	24.3 ± 1.8	44.7 ± 3.8 *

* $P < 0.001$ when compared to controls
** $P < 0.05$ when compared to controls

DISCUSSION

Plasma lipid peroxide levels are known to be increased in several pathological conditions (16 - 18). This increase can be due to massive cell death and lipid peroxidation or decrease in the activity of various scavenger systems (10). When evaluated together, significant increases in plasma lipid peroxide levels and activities of cytoplasmic glutathione transferases observed in this study suggest possible involvement of neuronal cell death.
The consequences of increased plasma lipid peroxide levels are mostly observed in susceptible target tissues. It has been suggested that erythrocyte membranes are labile to lipid peroxidation owing to their high content of polyunsaturated lipids (19). In addition, abnormal susceptibility of erythrocyte lipids to autoxidation are thought to reflect a similar abnormality in other organs and tissues as well as an impaired antioxidant protective mechanism (16 - 18).

Increased erythrocyte susceptibility to lipid peroxidation values observed in our study suggest that erythrocyte membrane integrity is impaired in Parkinson's disease. In addition, slightly decreased glutathione levels and significantly decreased glutathione peroxidase activities together point to the inefficiency of the erythrocyte antioxidant system in these patients. It seems reasonable to assume that these changes are all associated with each other.
While this study was in progress, Poirier and Barbeau (20) have reported that neither endogenous lipid peroxidation nor antioxidant activities were altered in erythrocytes of Parkinson's disease patients.We believe that this discrepancy arises from

differences in the methods used. It is very difficult to make reliable measurements of rather low levels of basal malonaldehyde content of erythrocytes, therefore we have decided that it would be more informative to use the procedure of Stocks et al. (12) where erythrocyte susceptibility to lipid peroxidation is measured under exogenous oxidative stress in the form of hydrogen peroxide.

Presently, we do not have enough evidence to suggest that increased plasma lipid peroxide levels have indeed originated from neurodegenerative changes in the substantia nigra. We can not exclude the possibility that L-DOPA taken by the patients may have accelerated production of reactive oxygen metabolites.

Recent views in Parkinson's disease treatment are in favor of preventing the disease by the use of antioxidants rather than curing the symptoms (21). We are currently using erythrocyte susceptibility to lipid peroxidation test and antioxidant levels as simple probes to determine whether use of L-DOPA is related to the observed changes and whether Parkinson's disease patients will benefit from antioxidant treatment.

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