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ORIGINAL PAPER

The Role of Oxidative Stress in Amyotrophic Lateral Sclerosis and Parkinson's Disease

Athan Baillet · Vanessa Chanteperdrix · Candice Trocmé · Pierre Casez · Catherine Garrel · Gérard Besson

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Abstract We examined oxidative stress markers of 31 patients suffering from ALS, 24 patients suffering from PD and 30 healthy subjects were included. We determined the plasma levels of lipid peroxidation (malondialdehyde, MDA), of protein oxidative lesions (plasma glutathione, carbonyls and thiols) and the activity of antioxidant enzymes i.e. erythrocyte Cu,Zn-Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px) and catalase. MDA and thiols were significantly different in both neurodegenerative diseases versus control population. A trend for an enhancement of oxidized glutathione was noted in ALS patients. Univariate analysis showed that SOD activity was significantly decreased in ALS and GSH-Px activity was decreased in PD. After adjusting for demographic parameters and enzyme cofactors, we could emphasize a compensatory increase of SOD activity in PD. Different antioxidant systems were not involved in the same way in ALS and PD, suggesting that oxidative stress may be a cause rather than a consequence of the neuronal death.

Keywords Oxidative stress · Reactive oxygen species · Neurodegeneration · Trace element · Amyotrophic lateral sclerosis · Parkinson's disease · Glutathione peroxidase · Superoxide dismutase

Abbreviations

ALS	Amyotrophic lateral sclerosis
EDTA	Ethylenediaminetetraacetic acid
GSH-Px	Glutathione peroxidase
MDA	Malondialdehyde
MOPS	3-N-morpholinopropaneslfonic acid
PD	Parkinson's disease
SOD	Cu,Zn-Superoxide dismutase

Introduction

Due to its high rate of oxygen utilization, high content of unsaturated lipids and relative lack of antioxidant enzymes, the brain is very vulnerable to free radical damage [1, 2]. Indeed free radicals are very toxic for motor and dopaminergic neurons. Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) are threatening neurodegenerative diseases that affect patients with a critical impact on their quality of life and on the health-care resources. Several studies suggested that oxidative stress plays a role in neurodegeneration [3, 4]. Post-mortem studies found a higher level of oxidative stress in the substantia nigra of PD patients [5, 6]. Moreover a significant decrease in the activity of the complex I of the mitochondrial electron

A. Baillet · G. Besson
Neurology Department, Hôpital A. Michallon,
38043 Grenoble Cedex 9, France

V. Chanteperdrix · C. Garrel
Biology Department, Hôpital A Michallon,
38043 Grenoble Cedex 9, France

C. Trocmé
Laboratory of Enzymology/DBPC, CHU Hôpital Michallon,
38043 Grenoble Cedex 9, France

P. Casez
Medical Information Unit, A. Michallon,
38043 Grenoble Cedex 9, France

A. Baillet (✉)
Grenoble University, Hôpital A. Michallon,
38043 Grenoble Cedex 9, France
e-mail: abaillet@chu-grenoble.fr

transport chain was reported in the substantia nigra [7, 8] and in platelets [9] of patients suffering from Parkinson's disease. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine can cause parkinsonism by an inhibition of complex I [10–12] and enhances lipid peroxidation in the substantia nigra. Finally a previous study proved that plasma from PD patients is more prone to peroxidation and contains higher levels of oxidative stress markers than in the control population [13].

Mutations in the Cu,Zn-Superoxide dismutase 1 (SOD1) gene account for 20% of familial ALS. Enhancement of oxidative damage markers was reported in ALS patients [3, 4, 14–18]. Furthermore signs of increased compensatory response to oxidative stress were found in patients with sporadic ALS [19].

Although there is increasing evidence of oxidative stress involvement in these two degenerative diseases, the lack of efficiency of antioxidant drugs raises the question of the role of oxidative stress in ALS and PD pathogenesis [20–25]. Oxidative damage may only be the result of the neurodegenerative process. This study was designed to compare oxidative lesions and anti-oxidative systems in ALS, PD and healthy populations in order to clarify the specific role of oxidative stress in these two diseases.

Experimental Procedure

Study Population and Sample Preparation

This study enrolled a total of 85 patients. Twenty four patients were diagnosed with sporadic PD. All of them underwent bilateral subthalamus stimulation. Thirty one definite sporadic ALS patients (according to the El Escorial criteria) were included in this study. Venous blood samples were collected very soon after diagnosis and before any treatment in the ALS group or during a control consultation at least 3 months after surgery in the PD group. The control group consisted of 30 healthy individuals. All of them were questioned and examined by an experienced neurologist to rule out a possible beginning of neurodegenerative disease. All participants gave their written consent prior to inclusion, according to the Declaration of Helsinki. The study design was approved by the local medical ethic committee. Every patient in the ALS and PD groups was treated in the Neurology Department of Grenoble Hospital, France. Red blood cells, obtained from blood samples, were evaluated for their antioxidant capacity by measuring the erythrocyte SOD, catalase, glutathione peroxidase (GSH-Px) activity and both oxidized and reduced glutathione in each group. Plasmas were frozen at −80°C for further measurement of oxidative stress markers (thiols, carbonyls, malondialdehyde (MDA)) and antioxidant enzyme cofactors (copper, zinc, selenium).

Measurement of Oxidative Markers in PD and ALS

MDA evaluates lipid peroxidation. The quantification method is based on the reaction of MDA with thiobarbituric acid followed by a reversed-phase high-performance liquid chromatography separation with fluorescence detection. A standard curve was performed with increasing concentrations of 1,1,3,3-tetraethoxypropane diluted in ethanol, in order to calibrate the fluorescence detector and to correlate fluorescence intensity to the MDA rate as previously described [26]. The concentration of plasma free thiols, which reflects the oxidative status of sulfhydril aminoacids, was evaluated according to the Ellman's test. This technic is based on the property of Ellman's reagent 5,5′-dithiobis (2-nitrobenzoic) acid to be reduced by free thiols in a yellow compound whose concentration was measured at 412 nm [27]. Carbonyls, which measure protein damage by free radicals, were evaluated through their reactivity with the 2,4-dinitrophenylhydrazine by forming a diphenylhydrazone compound that absorbs light at 380 nm. The carbonyl group content was calculated using an albumin standard curve and was referred to the protein concentration as previously described [28].

Evaluation of the Enzymatic Activity of SOD, GSH-Px and Catalase

Erythrocytes were first lyzed by a fourfold dilution in H_2O. SOD was extracted by a chloroform/ethanol solution (1:1, v/v) solution and after centrifugation (4,000 rpm, 25 min at +4°C), 50 µl of supernatant were added to 1,870 µl of the reaction buffer (50 mM cacodylic acid, 1 mM diethylenetriaminepentaacetic acid, 0.05 M Tris, pH 8.1) and 80 µl of 10 mM pyrogallol diluted in 36% HCl. Absorbance was measured at 420 nm. Values were converted to enzymatic units by reference to a SOD standard curve [29]. Specific Mn-SOD activity was obtained after inhibiting Cu,Zn-SOD activity with KCN. Specific Cu,Zn-SOD activity results from the difference between total-SOD activity and Mn-SOD activity.

Erythrocyte GSH-Px activity was determined by oxidation of reduced glutathione in presence of terbutyl hydroperoxyde. $NADPH_2$ was reduced into $NADP^+$ in presence of oxidized glutathione and glutathione reductase. Therefore GSH-Px activity was evaluated by the decrease of NADPH absorbance at 340 nm. Enzymatic reaction was performed at 25°C in a tube containing 900 µl of reaction buffer (1 mM ethylenediaminetetraacetic acid (EDTA), 4 mM NaN_3, 50 mM Tris, pH 7.6), 25 µl of a tenfold diluted hemolyzed erythrocytes, 20 µl of 0.15 M reduced glutathione, 20 µl of glutathione reductase (208 U/ml), 20 µl of 8.4 mM $NADPH_2$ and 20 µl of terbutyl hydroperoxyde 70% [30].