Antioxidant enzymes activity in subjects with Parkinson’s disease under L-DOPA therapy

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Abstract. Introduction: Age and oxidative stress represent two endogenous significant risk factors for developing Parkinson’s disease (PD). The selective loss of dopaminergic neurons in substantia nigra pars compacta (SNPC) leads to an increased turnover of dopamine by surviving neurons and an excessive production of reactive oxygen species (ROS) by enzymatic or chemical transformation of this neurotransmitter. Levodopa (L-DOPA), a dopamine precursor, represents the most effective medication for controlling the motor symptoms in PD. Some studies showed that this drug could enhance oxidative stress and may be responsible for the degeneration of residual dopamine neurons in subjects with PD.

Aim: Therefore, the aim of this study was to evaluate the activity of two key enzymes of the antioxidant defense system: superoxide dismutase (SOD) and glutathione peroxidase (GPx), and to investigate if these activities are correlated with the daily L-DOPA dose.

Material and methods: The study group included 18 PD patients receiving a mean L-DOPA dose of 352.94±175.41 mg/day and 16 healthy control subjects, age- and sex-matched. Results: SOD levels were significantly decreased in patients group compared to controls (1235.20±228.82 U/gHb vs. 1440.32±185.15 U/gHb; P=0.984). We did not find a significant correlation between the L-DOPA dose and the SOD or GPx activities, respectively (r=0.109, P=0.676; r=0.087, P=0.740). A positive and statistically significant correlation was observed between the Hoen & Yahe staging scale and the daily L-DOPA dose (r=0.683, P=0.03).

Conclusion: The results of this study indicate that there is an alteration of SOD activity in PD patients which is not correlated with the L-DOPA dose.

Key Words: Parkinson’s disease, L-DOPA, SOD, GPx, antioxidant protection.

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Introduction

The aetiology of Parkinson’s disease (PD), characterized by selective neuronal loss of dopaminergic neurons in the substantia nigra pars compacta (SNPC), is not yet fully elucidated despite of a great number of clinical and experimental studies performed (Kim et al 2015). The prevalence of this disease increases with age and oxidative stress has been reported to be one of the major causes in the onset and the progression of the neurodegenerative process (Pringsheim et al 2014). Levodopa (L-DOPA), the most prescribed and the most effective drug controlling the motor symptoms of the disease, may be responsible for accelerating the neuronal degeneration and the clinical progression of the disease (Olanow 2015). An analysis of literature data regarding the toxicity of L-DOPA suggests that this issue has not been fully resolved (Olanow 2015). The aim of this study was to assess the activity of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) in patients with PD, and to investigate a possible correlation between enzyme activities and daily L-DOPA dose.

Subjects and methods

The study group included 18 PD patients receiving a mean L-DOPA dose of 352.94±175.41 mg/day, hospitalized in the Neurology Clinic, Cluj-Napoca and 16 healthy control subjects, age- and sex-matched (60.83±8.3 years vs. 56.76±8.54 years). The patients were at a mean Hoehn and Yahr stage (Hughes et al 1992) of 2.63 ± 0.68. The informed consent was obtained from all subjects prior to their inclusion in the study. The research protocol was in agreement with the Declaration of Helsinki of the World Medical Association and was approved by the Ethical Committee of “Iuliu Hațieganu” University of Medicine and Pharmacy.

Venous blood was collected after overnight fasting, on EDTA for the assay of SOD activity, and on lithium heparin for the assay of GPx activity.

Erythrocyte superoxide dismutase activity assay

The whole blood (0.5 ml) was centrifuged at 590×g for 10 minutes and plasma was removed. The erythrocytes were washed three times with 0.9% NaCl solution and were stored at -80°C until analysis. SOD activity was assayed using a RANSOD
Whole blood glutathione peroxidase activity assay

The venous blood samples were stored at -80°C until analysis. The GPx activity was assayed using a RANSSEL kit (Cat. No. RS505, Randox lab. Ltd, Crumlin, UK) on a Cobas Mira Plus (Roche) analyzer according to the manufacturer’s instructions. This assay, based on the method of Paglia and Valentine (Paglia & Valentine 1967) requires cumene hydroperoxide as a substrate. The activity of GPx was expressed in U/gHb. Hemoglobin concentration was assayed with the Drabkin’s method (Drabkin & Austin 1952).

Statistical analysis

Statistical analysis was carried out using MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016). Correlation between quantitative variables was investigated using Spearman’s correlation coefficient (r) and was evaluated for statistical significance at an alpha level of 0.05. Since all investigated variables seemed to have originated from normal distributions, Student’s t-test for independent groups was used for hypothesis testing. Statistical significance has been defined for p<0.05.

Results

SOD levels were significantly decreased in patients compared to controls (P=0.008). There were not observed differences regarding GPx activity in PD subjects compared to controls (P=0.984) (Table 1). There was not find correlation between the daily L-DOPA dose and the SOD (r=-0.109, P=0.676) or the GPx activities (r=0.087, P=0.740). A positive and statistically significant correlation was observed between the Hoen & Yahe staging scale and the daily L-DOPA dose (r=0.683, P=0.03).

Table 1. Enzyme activities in PD subjects vs. controls

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>PD subjects</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/gHb)</td>
<td>1235.20±228.82</td>
<td>1440.32±185.15</td>
<td>0.008</td>
</tr>
<tr>
<td>GPx (U/gHb)</td>
<td>42.20±10.81</td>
<td>42.12±13.24</td>
<td>0.984</td>
</tr>
</tbody>
</table>

Discussions

Considerable evidence supports the assumption that oxidative stress plays an important role in the pathogenesis of PD (Kim et al 2015; Zhou et al 2008). Dopaminergic neurons in the substantia nigra (SN) are particularly vulnerable to oxidative stress due to the high metabolic demand, elevated iron concentration and lower glutathione (GSH) level on one hand, and the presence of dopamine and neuromelanin (NM) on the other hand (Smye & Smye 2013). In PD, the selective loss of dopaminergic neurons in the SNPC leads to an increased turnover of this neurotransmitter by surviving neurons (Spina & Cohen 1989) and an excessive production of reactive oxygen species (ROS) (Blesa et al 2015; Kim et al 2015). The metabolism of dopamine in SN represents a major source of ROS. Superoxide anion and hydrogen peroxide result through oxidative deamination of dopamine, catalyzed by monoamine oxidase. Moreover, a potent oxidant, the peroxynitrite, can be generated by the rapid reaction between superoxide anion and nitric oxide (Ceballos-Picot et al 2005). Some studies revealed that the activation of nitric oxide synthase and the overproduction of peroxynitrite ion may contribute to neurodegeneration in PD (Ebadi & Sharma 2005). The elevated level of free iron in the SN of PD patients may also lead to the disturbance of the redox state (Oakley et al 2007; Haake et al 2007). Hydrogen peroxide can react with the free iron and generates cytotoxic hydroxyl radical yielding lipid peroxidation (Hauser & Hastings 2013). In this context, increased lipid peroxidation and decreased GSH level in PD subjects were reported by Sharma (2008). The GSH depletion may lead to mitochondrial dysfunction, probably due to the inactivation of complex I, and consecutively to the increase of ROS production. In this respect, a correlation was reported between the GSH level and the severity of PD (Smye & Smye 2013; Bharath & Andersen 2005). Dopamine autooxidation and a looser association between iron and NM may result in an increased production of free radical species (Ceballos-Picot et al 2005; Zecca et al 2001). L-DOPA therapy might be a source of cytotoxic ROS because dopamine (formed by the L-DOPA decarboxylation) is easily transformed chemically or enzymatically (Konip 1993; Zucca et al 2014). It was suggested that, as a consequence of ROS formation, L-DOPA might be toxic (Olanow 2015). In order to verify this hypothesis, in this study we assayed the activity of antioxidant enzymes SOD and GPx and evaluated the correlation between enzyme activities and L-DOPA dose.

SOD catalyzes the dismutation of superoxide anion into molecular oxygen and hydrogen peroxide, thus providing a primary defense against ROS. This enzyme is present at high levels of constitutive expression in neuromelanin-containing dopaminergic neurons of the SN (Bergeron et al 1996). It is a key antioxidant but also a highly prooxidant enzyme because it generates hydrogen peroxide. Different reports on erythrocyte SOD activity in PD subjects showed divergent results. Some studies reported an enhanced SOD activity (as an adaptive response to the increased oxidative stress) (Serra et al 2001) and others, on the contrary, reported low levels (Urakami et al 1992; Gatto et al 1996; Bostanjopoulou et al 1997; Ibara et al 1999; Abraham et al 2005; Sunday et al 2014). We suppose that the significantly decrease of SOD activity observed in this study could be due to an increasing and persisting production of ROS and might occur either via direct oxidative damage of the enzyme, or via alteration of SOD gene expression mediated by ROS, or both. GPx is a selenoenzyme that catalyzes the removal of hydrogen peroxide and the reduction of lipid peroxides to the corresponding alcohols. The enzyme has also a peroxynitrite reductase activity (Prabhakar et al 2006; Sies et al 1997). Damier et al. reported that GPx is localized exclusively in the glial cells of the midbrain (Damier et al 1993); for this reason, the enzyme removes with difficulty the hydrogen peroxide formed in neurons (Ceballos-Picot et al 2005).
In this study, no change was observed in GPx activity in PD patients vs. controls. The results are in agreement with those reported by Sudha et al (2003) for the erythrocyte GPx activity. An unchanged GPx activity was also reported in postmortem brain tissue of PD subjects (Jenner et al 1992). Nevertheless, some authors reported a decrease of enzyme activity in PD patients (Abraham et al 2005; Sunday et al 2014).

The results of this study are also in agreement with those of Bostanjopoulou et al (1997) who reported a significant decrease of erythrocyte SOD activity in PD subjects, and no relationship between L-DOPA treatment and the enzyme activity. The small size of the investigated groups may be a limitation of the current study. Further studies should be performed on a larger sample of PD subjects, by coupling the measurement of the activity of antioxidant enzymes with the assay of malondialdehyde and the ratio between reduced and oxidized glutathione that are recognized to be more reliable indicators of oxidative stress.

Conclusions
The results of the study indicate an alteration of the SOD activity which is not correlated with L-DOPA dose. Further investigations on a larger sample of PD subjects are required to confirm the result of the present study.

References


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