Plasma lipid peroxidation in sporadic Parkinson’s disease. Role of the L-dopa

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Abstract

Oxidative stress plays an important role in the pathogenesis of neurodegenerative diseases, such as Parkinson’s disease (PD). There are several methods to measure oxidative stress, being lipid peroxidation (LPO) one of the most frequently used. Endogenous plasma LPO was determined by a spectrofluorimetric method in fifty two patients with sporadic PD and in forty controls. To know the maximum capacity of lipids to peroxidate, LPO was also measured after co-incubation with Fe 2+/H 2O2 (exogenous LPO). All PD patients were taking L-dopa and the effect of this treatment on LPO levels was additionally studied. Urine catecholamines and their main metabolites were also analyzed, and their possible correlation to LPO statistically studied. Endogenous plasma LPO levels were 33% higher in PD group than in control group (P<0.001). Exogenous plasma or oxidizability was also higher in PD patients compared to controls (20%, P<0.05). The intake of L-dopa was negatively dose-related to endogenous and exogenous plasma LPO. In conclusion, plasma of PD patients has elevated levels of LPO and also is more prone to peroxidation than that in the control group. The results also suggest an antioxidant effect of L-dopa.
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Keywords: Parkinson’s disease; Oxidative stress; L-dopa; Catecholamines; Lipid peroxidation

1. Introduction

Oxidative stress is believed to be involved in the pathogenesis of various diseases including atherosclerosis, diabetes mellitus, epilepsy, inflammatory and neurodegenerative diseases, and cancer [1–5]. The increase of free radicals produces lipid peroxidation, one of the major causes of cell membrane damage that eventually leads to degeneration of cells [4].

Parkinson’s disease (PD) is a common disabling neurodegenerative and progressive disorder characterized by rigidity, tremor, and motor disturbances. The pathogenesis of PD is not completely understood, but oxidative stress seems to be one of the main etiological factors [6]. In PD, the pathological death of dopaminergic neurons in the substantia nigra (SN) pars compacta leads to less availability of dopamine in the striatum. Since the oxidative metabolism of dopamine generates cytotoxic free radicals, neurons of SN are particularly vulnerable to oxidative stress. Dopamine can be oxidized by either monoamine oxidase or undergo autooxidation to generate hydrogen peroxide [7]. H 2O2 can damage the neuron directly or indirectly through the
formation of hydroxyl radicals in presence of ferrous ions by the Fenton reaction [8,9]. Not only dopamine, but also the dopamine metabolite neuromelanin, found within the SN neurons, promotes site-specific accumulation of iron, thereby potentiating iron-induced LPO, and consequently cell death [10,11]. Thus, free radicals are one of the most important agents responsible of SN neuronal degeneration.

Although the role of peroxidation in SN is well established, the significance of peripheral oxidative stress in PD is still unclear and the results are apparently contradictory. While some studies showed an increase of oxidative stress [12,13], other authors described no changes [14]. Differences in the results may be due to differences in the methods used to measure systemic oxidative stress. We have previously suggested that the oxidation of LDL-lipoprotein and/or erythrocytes may be an indicative of systemic oxidative stress [9,15]. However, these studies provide only a limited picture, because these approaches fail to evaluate the roles of pro-oxidants and anti-oxidants factors in plasma which play an important role on the oxidative stress regulation. Plasma oxidation may be more biologically relevant than other such as LDL and/or erythrocyte’s oxidation since water-soluble antioxidants such as ascorbic acid, uric acid and other plasma constituents would also be taken into account.

In addition, L-dopa is one of the most used drug in the treatment of PD. In the CNS, L-dopa is quickly transformed in dopamine and both may be relevant in oxidative stress. The importance of L-dopa on systemic oxidative stress in PD patients remains unresolved. Peripheral oxidation status in PD might be affected by the possible effect of L-dopa treatment. L-dopa seems to exert toxic effects on cultured dopaminergic neurons [16]. However, there is no evidence from in vivo studies suggesting that L-dopa treatment damages SN neurons in PD. On the other hand, while Martignoni et al. [17] have showed that L-dopa therapy increased the concentration of 2,3 dihydroxybenzoate in platelets of PD patients, other studies have described no correlation between the dose of L-dopa and basal plasma oxidative status measured as % CoQ-10 [18], serum malonilialdehyde [19], or blood hydroxyl radicals and plasma [14].

The aim of this study was to evaluate the oxidative stress and the susceptibility of plasma oxidation in PD patients. Additionally, the possible correlation among L-dopa, catecholamines and its main metabolites and the oxidative status in PD was also studied.

2. Materials and methods

Fifty two patients were diagnosed of sporadic PD (PD group). All patients were under treatment in the Alteration Movement Unit of the University Hospital of Granada, Spain. The control group consisted of a randomized group of forty subjects, showing acute or chronic osteoarticular pathology, with similar distribution of age and sex. All of them were examined and questioned by an experienced neurologist to rule out signs and symptoms suggestive of early PD, and they had no known relatives with PD. On the other hand, PD was diagnosed by at least two neurologists, following the criteria of the Brain Bank of the Society of Parkinson’s disease of United Kingdom [20]. To assess PD patients a clinic interview, demographic data, clinical history (evolution time, symptoms, half of the body more affected at the beginning), step of PD (Hoehn and Yahr score) [21], and UPDRS (Unified Parkinson’s Disease Rating Scale) [22] were used. This study was approved by the Ethical Committee for Human Research of the University Hospital of Granada.

All PD patients were taken L-dopa and the duration of PD was 87.88±8.16 months since diagnosis. The Hoehn and Yahr stage were: stage 1, 6 patients; stage 2, 19 patients; stage 3, 21 patients; stage 4, 5 patients and stage 5, 1 patient. The UPDRS values were: UPDRS (I)=3.58±0.38; UPDRS (II)=14.26±1.44 and UPDRS (III)=23.32±1.76 (mean±S.E.M.).

2.1. Analysis of catecholamines

After column extraction, concentrations of noradrenaline, adrenaline, dopamine, vanil mandelic acid, 5-hydroxyindol acetic acid, and homovanillic acid in urine collected during 24 h were determined by HPLC with electrochemical detection by commercially available kits (N.S. Lab, S.L., Seville, Spain).

2.2. Determination of plasma lipid peroxides

To determine lipid peroxide concentration, plasma was collected in tubes containing EDTA as an anticoagulant and stored at – 80 °C until analysis. Cholesterol and triglycerides were measured. Lipid peroxides were determined in duplicate both in the basal state and after co-incubation with hydrogen peroxide and ferrous salt to generate hydroxyl radicals by the Fenton reaction. A modification of the assay of Yagi [23] was used. Three modifications were made to improve the specificity of the plasma LPO measurement. First, water-soluble substances, which also react with TBA to yield the same product as LPO, were removed by including a phosophotungstic acid/sulfuric acid step to precipitate lipids with proteins. Second, interference from bilirubin was prevented by reading the fluorescence at 553 nm rather than at 532. Finally, sialic acid interference was eliminated by adding acetic in the TBA reagent. The detailed method has been published elsewhere [9]. The results obtained with this method have been shown to correlate strongly with high-pressure liquid chromatography methods for the determination of LPO levels (r=0.85). The intra-assay and inter-assay coefficients of variation for measured lipid peroxide concentrations were 4.0% and 4.2%, respectively, in our laboratory.
2.3. Statistical analysis

Statistical analysis was performed using one way ANOVA followed by Bonferroni, Games-Howell, or Dunnet t test where appropriate. Correlations between L-dopa and LPO end or LPO exo were analyzed by Pearson correlation.

3. Results

There were not significant differences in age and sex between groups. Both, control and PD groups were normolipemic: control group, cholesterol 165.85 ± 6.00 mg/100 mL and triglycerides 179 ± 10.38 mg/100 mL; PD group, cholesterol 209.08 ± 6.48 mg/100 mL and triglycerides 130.69 ± 9.73 mg/100 mL. Although all these values were within the range of normality, control group had cholesterol levels lower than in the PD group (P < 0.001) and triglycerides higher than in the PD group (P < 0.001). Table 1 shows medication regime of PD patients. All PD patients were taking L-dopa together with the medication indicated in the table. Values represent mean ± S.E.M. n = number of subjects taken medication.

Table 1
Medication regime of PD patients

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-dopa (52)</td>
<td>596.57 ± 39.35</td>
</tr>
<tr>
<td>Pramipexole (18)</td>
<td>1.31 ± 0.85</td>
</tr>
<tr>
<td>Entacapone (11)</td>
<td>645.45 ± 175.30</td>
</tr>
<tr>
<td>Selegiline (11)</td>
<td>8.64 ± 2.33</td>
</tr>
<tr>
<td>Pergolide (10)</td>
<td>2.04 ± 1.05</td>
</tr>
<tr>
<td>Ropinirole (5)</td>
<td>9.00 ± 8.48</td>
</tr>
<tr>
<td>Bromocripline (2)</td>
<td>10.00 ± 7.07</td>
</tr>
<tr>
<td>Lisuride (1)</td>
<td>0.40 ± 0.00</td>
</tr>
<tr>
<td>Am-triptiline (5)</td>
<td>12.50 ± 0.00</td>
</tr>
<tr>
<td>Nor-triptiline (1)</td>
<td>12.50 ± 0.00</td>
</tr>
<tr>
<td>Maprotyline (1)</td>
<td>10.00 ± 0.00</td>
</tr>
</tbody>
</table>

All PD patients were taking L-dopa together with the medication indicated in the table. Values represent mean ± S.E.M. n = number of subjects taken medication.

Table 2
Plasma concentration of lipid peroxides (LPO) in controls and PD patients

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 40)</th>
<th>PD (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO end (μmol/L)</td>
<td>2.80 ± 0.12</td>
<td>3.19 ± 0.16***</td>
</tr>
<tr>
<td>LPO exo (μmol/L)</td>
<td>3.90 ± 0.17</td>
<td>4.70 ± 0.28*</td>
</tr>
</tbody>
</table>

LPO end: Plasma LPO in absence of FeSO4 and H2O2. LPO exo: Plasma LPO in presence of FeSO4 and H2O2. Mean ± S.E.M. of two separate experiments. ns: not significant; *P < 0.05; ***P < 0.001. Statistical analysis was performed using one way ANOVA followed by Bonferroni and bilateral Dunnet t test.

Table 3
Concentrations of noradrenaline, adrenaline, dopamine, vanil mandelic acid, 5-hydroxyindol acetic acid, and homovanillic acid in urine collected during 24 h

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 17)</th>
<th>PD (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline (μg/L)</td>
<td>25.90 ± 3.76</td>
<td>37.98 ± 4.08***</td>
</tr>
<tr>
<td>Adrenaline (μg/L)</td>
<td>3.03 ± 0.60</td>
<td>13.25 ± 4.05***</td>
</tr>
<tr>
<td>Dopamine (μg/L)</td>
<td>1886.20 ± 1757.21</td>
<td>11,141.91 ± 2213.18***</td>
</tr>
<tr>
<td>Vanil mandelic (mg/L)</td>
<td>2.46 ± 0.33</td>
<td>4.33 ± 0.41***</td>
</tr>
<tr>
<td>HIAA (mg/L)</td>
<td>3.08 ± 1.33</td>
<td>3.82 ± 0.52*</td>
</tr>
<tr>
<td>HVA (mg/L)</td>
<td>2.46 ± 0.24</td>
<td>37.09 ± 3.42***</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. of two separate experiments. ns: not significant; *P < 0.05; ***P < 0.001. Statistical analysis was performed using one way ANOVA, followed by Bonferroni and Games Howell tests.

Table 3 shows the concentrations of noradrenaline, adrenaline, dopamine, vanil mandelic acid, 5-hydroxyindol acetic acid, and homovanillic acid in urine collected for 24 h. Except noradrenaline and adrenaline, all other catecholamine and metabolites increased significantly in PD group, between 1.23 and 6.83 μmol/L and LPO exo between 2.27 and 7.96 μmol/L.

Fig. 1. Linear regression showing the negative relationship between L-dopa dose (mg/day) and LPO end (A) and LPO exo (B). Graphics shows mean prediction with 95% of confidence (curves) and individual prediction interval, also with 95% of confidence.
composed of patients taken L-dopa at dose individually adjusted. The highest increase was observed in plasma dopamine.

The correlation between antiparkinsonian medication, catecholamines and their metabolites and LPO<sub>endo</sub> and LPO<sub>exo</sub> was also assessed. L-dopa was negatively correlated to LPO<sub>end</sub> (<i>P</i>&lt;0.01) and LPO<sub>exo</sub> (<i>P</i>&lt;0.01) (Fig. 1A and B). Dopamine and adrenaline were also negatively correlated to LPO<sub>end</sub> (<i>P</i>&lt;0.01 and <i>P</i>&lt;0.05, respectively) and LPO<sub>exo</sub> (<i>P</i>&lt;0.01 and 0.05, respectively). Homovanillic acid was negatively correlated to LPO<sub>end</sub> (<i>P</i>&lt;0.05).

4. Discussion

In the present study, we found raised endogenous plasma LPO concentrations (13.9%, <i>P</i>&lt;0.001) (Table 2) in PD patients, suggesting that they are chronically under systemic oxidative stress. These findings are in agreement with other studies in PD, showing increases in systemic oxidative stress, measured in plasma as LPO levels [12,13]; conjugated dienes [24] and coenzyme Q10 oxidized [18]. On the contrary, other study reported no significant changes in serum level of malonildialdehyde (MDA) in PD patients [14]. In our study, all PD patients were under L-dopa treatment, and this drug might be responsible for changes in plasma oxidation ex-vivo. Despite that in vitro studies have demonstrated the potential of L-dopa to exert toxic effects on cultured dopaminergic neurons [16], there is little or no evidence from in vivo models suggesting that L-dopa treatment damage nigral neurons in PD [31]. Therefore, it is believed that L-dopa may increase free radicals generation by increasing dopamine oxidation and thus, facilitating PD progression. It is possible that L-dopa treatment (by itself or by their metabolites) may also affects peripheral endogenous and/or exogenous LPO levels. Our results show that the intake of L-dopa correlates negatively with LPO<sub>end</sub> in a dose-dependent way (<i>y</i>=1.41−0.0001<i>x</i>; <i>r</i>=0.51, <i>P</i>&lt;0.01 ) (Fig. 1A) and moreover, we also found a negative correlation between L-dopa and LPO<sub>exo</sub> (Fig. 1B). These results, obtained through the ex vivo model system [9], may indicate that L-dopa can act as antioxidant in plasma and thus ameliorate PD progression. Several studies described a non significant correlation between L-dopa dose and basal oxidative status, measured as plasma % CoQ-10 [18], serum MDA [19], blood hydroxyl radicals and plasma SOD [14]. Except in the case of L-dopa, we did not find any significant correlation between LPO and medication regime. Experimentally, selegiline (deprenyl) showed antioxidant properties. Although it may have anti-oxidant effects in PD patients, the low number (11) of patients that were taken selegiline in this study did not permit to find out any difference. Like L-dopa, dopamine or others catecholamine metabolites can affect oxidative stress under experimental conditions. We found elevated concentrations of dopamine, vanil mandelic acid, 5-hydroxyindol acetic acid, and homovanillic acid in urine of PD patients, being dopamine levels the highest. Only dopamine and adrenalin levels were negatively correlated to LPO<sub>end</sub> (<i>y</i>=1.34−0.111<i>x</i>; <i>r</i>=0.40, <i>P</i>&lt;0.05;
y = 1.61 + −0.02x; r = 0.48, P < 0.01, respectively). Our findings agree with those of Scigliao et al. [32] who showed an increase of plasma bilirubin, a potent antioxidant [33] in PD patients under L-dopa treatment compared to untreated parkinsonians and controls. In opposition, other authors have suggested a potential prooxidative role of L-dopa [24].

Therefore, the present results could indicate that L-dopa, dopamine and adrenalin may act as antioxidants in plasma, and thus they might ameliorate peripheral collateral effects that accompanies PD progression. In contrast to others investigations, our study measured plasma susceptibility to an exogenous OH·, which actually reflects the resistance of plasma toward oxidation and therefore provides information about the entire oxidative status of plasma in PD patients. As a result, L-dopa treatment can lead to a decreased systemic free radicals generation associated to this process and subsequently, to diminish plasma endogenous and exogenous LPO levels. These findings point out that there is a reduction in the concentrations of plasma LPO in PD patients treated with L-dopa in a dose-related manner.

Taken together the above mentioned data, it can be concluded that plasma from PD patients is under oxidative stress as evidenced by increased basal LPO. Our results support the involvement of systemic oxidative stress in pathogenesis of PD and provide additional information about the role of systemic L-dopa as antioxidant. We suggest that the progressive impairment of PD that has been related to L-dopa treatment is not mediated by oxidative processes, at least at peripheral level.

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References


