Glutathione peroxidase in early and advanced Parkinson’s disease

P Johannsen, G Velander, J Mai, E B Thorling, E Dupont

Abstract
A defective antioxidant scavenging system plays a major role in one of the theories of the pathogenesis of Parkinson’s disease. The aim of this study was to investigate whether there is a general difference in antioxidant activity between early and advanced cases of Parkinson’s disease. Twenty five recently diagnosed patients, without any clinical fluctuations (group A), and 25 patients in a late phase of the disease with severe fluctuations in response to levodopa therapy (group B) were included in the study. Erythrocyte glutathione peroxidase was determined as a measure of antioxidant activity and significantly lower values were found in group B than in group A. Regression analyses in groups A and B showed significant correlation between glutathione peroxidase and duration of disease, but not between glutathione peroxidase and age of patients.

The pathogenesis of the progressive loss of dopaminergic neurons in the substantia nigra (SN), characteristic of Parkinson’s disease (PD) has not yet been revealed. One of the theories is that PD patients have a defective antioxidant system, leading to an increased formation of highly reactive free oxygen radicals (O
ds). These are able to react with, for example, polyunsaturated fatty acids of the cell membranes, thereby causing cells to lyse and die. The free radical formation is a result of monoamineoxidase activity in the SN, and of the oxidation of dopamine via 6-hydroxydopamine (6-OHDA). Furthermore, levodopa used in the treatment of PD may autodestruct and give rise to an increased production of O
ds in the CNS of these patients. Free radicals are usually eliminated by the antioxidant system which may be divided into a hydrophil and a lipophil part. The hydrophil part includes molecular reducing substances such as vitamin C and the enzymes superoxide dismutase, catalase and the selenium-dependent glutathione peroxidase (GSH-px). The lipophil part consists mainly of the carotenoles and the tocopherols. There have been several studies on the antioxidant system in PD. Perry et al found a significantly reduced glutathione (GSH) content in the SN in PD patients compared with other parts of the brain. Other studies have shown that the GSH-px activity in SN of PD patients was significantly reduced compared with controls.

Studies on erythrocytes (rbc) by Poirrier and Barbeau did not find any reduction in rbc-GSH, rbc-GSH-px, superoxide dismutase and catalase activity, while a study by Killinc et al concluded that the rbc-GSH-px activity was significantly reduced in Parkinsonian patients compared with controls. Thus it is still undecided whether the reduced antioxidant activity found in the SN in PD patients is part of a general aberration or is of local origin.

The main problem in the management of PD is the long-term complications of levodopa treatment, known as the late Parkinsonian syndrome. It consists of highly disabling fluctuations in motor performance and occurrence of abnormal, involuntary movements. Previous studies on the antioxidant system in PD have not differentiated between early and advanced cases. The possibility that levodopa therapy, via 6-OHDA, might enhance the destruction of the SN (4) makes it relevant to study the possible correlation between the progression of PD and the antioxidant system.

The aim of this study was to determine whether there is a difference in the antioxidant activity, measured by rbc-GSH-px activity and selenium in plasma and erythrocytes, between Parkinsonian patients in the early stages of the disease and patients with advanced disease.

Patients and methods
Fifty patients with idiopathic PD, selected according to their stage of disease, were included in the study. Forty were outpatients and the rest inpatients admitted for adjustment of anti-Parkinsonian medication and treatment of motor fluctuations at our department between March and July of 1988. Twenty five were recently diagnosed patients with a smooth response to levodopa (group A), and 25 were in the late stages of the disease with pronounced fluctuations of the therapeutic response (group B). Informed consent was obtained from all patients. The Helsinki II declaration was followed and the study was approved by the local ethical committee.

On the day of examination the patients were rated according to the Unified Parkinson’s Disease Rating Scale (UPDRS). Age and duration of disease for the two groups, as well as their UPDRS-scores, are shown in table 1.
Table 1 Age of patients, duration of disease and UPDRS-score. Results are expressed as mean (SD), range in brackets and p-value from Mann-Whitney test between group A and group B.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age (years)</th>
<th>Duration of disease (years)</th>
<th>UPDRS-score (9-147)</th>
</tr>
</thead>
<tbody>
<tr>
<td>group A</td>
<td>25</td>
<td>63.2 (7.8)</td>
<td>3.5 (1.9)</td>
<td>18.6 (11.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(50-77)</td>
<td>(0-8)</td>
<td>(4-9)</td>
</tr>
<tr>
<td>group B</td>
<td>25</td>
<td>68.0 (5.8)</td>
<td>13.2 (5.1)</td>
<td>59.5 (22.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(57-78)</td>
<td>(4-24)</td>
<td>(21-109)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The patients were interviewed about their daily food-intake and -habits, and it was noted whether they had taken supplementary vitamins and minerals, especially selenium supplementation. Nine patients in group A and six in group B supplemented with selenium.

A fasting venous blood sample was drawn (40 ml). The blood sample was immediately placed on ice and processed at once at the laboratory of the Department for Diet and Cancer, Danish Cancer Society. Erythrocyte glutathione peroxidase (rbc-GSH-px) was measured using the method of Paglia et al.18 with ter-butylhydroperoxide (from Merck art 820244) as substrate. Plasma- and erythrocyte-selenium were measured according to the method of Watkinson,19 as described by Thorling et al.20 In group A, one measurement of rbc-selenium from a patient, taking selenium supplementation, was lost during processing.

The statistical analyses were performed by the Statistical Program for Social Science (SPSS-PC). For analyses of significance the Mann-Whitney nonparametric test was used.

Results
In Table 1 the differences in age of the patients, duration of the disease and the UPDRS-scores are showed.

The rcb-GSH-px level was 30.4 per cent lower in patients of group B (table 2) compared with group A (p < 0.0001) (n = 50). When excluding those taking supplementary selenium the reduction was 22.4 per cent, but still significant (p = 0.002) (n = 35). Plasma-selenium was significantly lower in group B than in group A, 12.5 per cent (p = 0.03) (table 3). When omitting those taking selenium supplementation, however, there was no significant difference between the two groups. Nor did rbc-selenium show any significant differences between the two groups (table 4).

Even though patients from group B took supplementary selenium they did not have an increased rbc-GSH-px level (table 2), or rbselenium level (table 4). Only plasma-selenium rose by 13.9 per cent (table 3, fig 1), though it is not considered statistically significant. The patients in group A who took supplementary selenium rose significantly both in rbc-GSH-px by 30.1 per cent (p = 0.015) (table 2, fig 1) and in plasma-selenium by 25.9 per cent (p = 0.011) (table 3, fig 1), while the 11.2 per cent rise in rbc-selenium in this group from 184 μg/l cells to 205 μg/l cells (table 4) was not significant (p = 0.48). The three oldest patients in group A, who took supplementary selenium, showed very high levels in rbc-GSH-px, indicating that the stage of disease may be of more importance than age (fig 2).

As expected a significant correlation between levels of rbc-GSH-px and plasma-selenium was found in group A (r = 0.64; p < 0.0005).21,22 This did not apply to group B (r = 0.25; p = 0.22). For the patients without selenium supplementation, no significant correlation between rbc-GSH-px and age was found in either group A or B (fig 1), but a significant correlation between rbc-GSH-px and duration of disease was found within the two individual groups (fig 2).

Erythrocyte-GSH-px shows no significant correlation to the UPDRS-score in group A (p = 0.66) or in group B (p = 0.20).

A significant correlation between plasma-selenium and age was shown in group A in patients without selenium supplementation but not between plasma-selenium and duration of disease (table 5). The opposite was seen in group B in which a significant correlation between plasma-selenium and duration of disease was seen but not between plasma-selenium and age. As shown in table 6, rbc-selenium has no significant correlation with either age or duration of disease in group A, but in group B the results are the same as those for plasma-selenium.

The interview on food habits revealed that there was no difference between the two groups on the estimated energy intake, quality of food or adequacy of nutritional elements that could influence the GSH-px level. The only exception was the selenium supplementation used by 15 of the patients. There was no difference between the sexes concerning any of the parameters.

Discussion
The aim of this study was to investigate whether there is a difference in antioxidant activity between PD patients at the early stage of their disease and those suffering from advanced PD. We believe that this aim has been fulfilled.

Parkinsonian patients in the late phase of the disease have a lower antioxidant activity as...
Glutathione 

supplementation: Group A: correlation coefficient: $r = -0.3117$ probability: $p = 0.24$ ($n = 16$). Group B: correlation coefficient: $r = -0.0721$ probability: $p = 0.75$ ($n = 19$).

The study cannot determine whether the reduction in rbc-GSH-px is a part of the pathogenesis or a result of the disease or the drug treatment. A clue is provided by fig 2 suggesting that the reduction in rbc-GSH-px is a result of the disease or drug treatment due to the steady decline during the years of disease.

Table 4 Erythrocyte-selenium (µg/l cells). Results are expressed as mean (SD) and p-value from Mann-Whitney test between group A and group B

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Patients without selenium supplementation</th>
<th>Patients with selenium supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 24)</td>
<td>191 (50)</td>
<td>184 (45)</td>
<td>205 (60)</td>
</tr>
<tr>
<td></td>
<td>(n = 16)</td>
<td>(n = 8)</td>
<td></td>
</tr>
<tr>
<td>group B</td>
<td>176 (40)</td>
<td>176 (40)</td>
<td>176 (41)</td>
</tr>
<tr>
<td>(n = 25)</td>
<td>(n = 19)</td>
<td>(n = 6)</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
<td></td>
<td>0.57</td>
</tr>
</tbody>
</table>

Table 5 Regression analyses of pl-selenium with different variables

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 16)</th>
<th>Group B (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma-selenium with:</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>-0.1453</td>
<td>0.59</td>
</tr>
<tr>
<td>Age of patients</td>
<td>-0.6909</td>
<td>0.003</td>
</tr>
</tbody>
</table>

All patients without selenium supplementation. $r = $ correlation coefficient. $p = $ probability.

Table 6 Regression analyses of rbc-selenium with different variables

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 16)</th>
<th>Group B (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte selenium with:</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>-0.3270</td>
<td>0.40</td>
</tr>
<tr>
<td>Age of patients</td>
<td>-0.3706</td>
<td>0.16</td>
</tr>
</tbody>
</table>

All patients without selenium supplementation. $r = $ correlation coefficient. $p = $ probability.

So far there has been no consensus on the activity of free radical protecting enzymes in PD. According to Ambani et al., the activity of catalase and peroxidase, the enzymes scavenging hydrogen peroxide, was decreased in SN from PD patients. Perry et al. found virtually absent glutathione, the substrate for GSH-px, in Parkinsonian SN. Also studies by Kish et al. confirmed a reduced activity of GSH-px in SN in the brains from PD patients. However, Poirier and Barbeau and Marttila et al. did not find alterations in free radical protecting enzymes in peripheral blood from PD patients, indicating no general defect in these enzymes.

This contrasts with results reported by Kilinc et al. who found decreased GSH-px and glutathione in erythrocytes from PD patients. One explanation as to why no alteration could be detected might be that their studies were based on early PD patients or a mixture of early and late cases, while those who found alterations based their studies on patients in the late phase of Parkinson's disease. Also differences in analytical and sampling procedures may account for some of the differences.

We find the difference between early and late PD patients convincing. We therefore conclude that some general defect in the free radical protecting enzymes does develop over the years of Parkinson's disease. This is supported by the observation that early patients, even those that are elderly, supplementing themselves with selenium, have a high level of rbc-GSH-px, while the late patients, even young ones, seem to have lost the ability to increase their rbc-GSH-px by selenium ingestion and thus have less free

Figure 1 Erythrocyte GSH-px plotted against age of patients. Group A without selenium, $n = 16$. Group A with selenium, $n = 9$. Group B without selenium, $n = 19$. Group B with selenium, $n = 6$. Patients without selenium supplementation: Group A: correlation coefficient: $r = -0.3117$ probability: $p = 0.24$ ($n = 16$). Group B: correlation coefficient: $r = -0.0721$ probability: $p = 0.75$ ($n = 19$).

Figure 2 Erythrocyte GSH-px plotted against duration of disease. Group A without selenium, $n = 16$. Group A with selenium, $n = 9$. Group B without selenium, $n = 19$. Group B with selenium, $n = 6$. Patients without selenium supplementation: Group A: correlation coefficient: $r = -0.5406$ probability: $p = 0.03$ ($n = 16$). Group B: correlation coefficient: $r = -0.4650$ probability: $p = 0.04$ ($n = 19$).
radical protecting capacity. It is possible that this loss is due to increased oxygen stress induced by the levodopa therapy. This would be in accordance with the impression that combination therapies with dopamine agonists and thereby reduced amounts of levodopa may postpone the development of fluctuations in therapeutic response and of the “on-off syndrome”.25

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Neurological stamp

Avicenna or Ibn Sina (980–1036 AD)

He was court physician, vizier to different caliphs and physician in chief to the celebrated hospital in Baghdad, and translator of Galen. Avicenna wrote a gigantic medical tome, and had a great reputation in his time. He gave us the term “vermis” and “tailed nucleus” from which was derived “caudate nucleus”.

He described meningitis which he considered to be inflammation or tumour of the envelopes of the brain, and distinguished this from secondary meningitis. He knew about the pupil and its movement, of six motor muscles for the globe, and of central and peripheral types of facial weakness. Avicenna defined apoplexy as “loss of sensibility and movement following an occlusion seated within the brain in those places traversed by the nervous influx of sensibility and motricity.”

This Russian stamp was issued in 1980 to commemorate one thousand years of his birth. (Stanley Gibbons 5022, Scott 4852).

L.F. HAS