8-OHdG in Cerebrospinal Fluid as a Marker of Oxidative Stress in Various Neurodegenerative Diseases

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Introduction

There is increasing evidence that oxidative damage is involved in the pathogenesis of various neurodegenerative disorders, including amyotrophic lateral sclerosis [1, 2], Huntington’s disease [3], Parkinson’s disease (PD) [4, 5] and Alzheimer’s disease (AD) [6–8].

The nucleic acid can be damaged by reactive oxygen free radicals, which are generated in cellular metabolic pathways and by exposure to environmental oxidants. Highly reactive oxygen species (specifically the hydroxyl radical) attack DNA, leading to the hydroxylation of DNA and RNA [9–12]. Among the multiple products of nucleoside oxidation, 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-hydroxyguanosine (8-OHG) are 2 of the most prominent and best characterized [10, 11].

Brain tissue shows a higher susceptibility to oxidative stress due to free radicals, which is caused by insufficient content of antioxidants, the considerable content of polyunsaturated fatty acid side chains in the cell membrane lipid layer, and by its high oxygen consumption rate [13–15]. Moreover, ageing is associated with increased production of free radicals, and promoters of oxidative DNA damage have been found to be increased in the cerebral tissue of healthy aged subjects [16] as well as patients with AD [6, 17].
Several studies have found increased levels of 8-OHdG in the damaged neuronal populations of postmortem brains in patients with AD [6, 7], Huntington’s disease [3] and amyotrophic lateral sclerosis [1], as well as increased 8-OH in the substantia nigra of PD patients [18, 19]. In addition to this brain-associated damage, high levels of 8-OHdG have also been demonstrated in peripheral tissues in AD [20], suggesting a systemic increase in oxidative nucleic acid damage in neurodegenerative diseases. In PD, oxidative stress has been suggested to play a pivotal role in disease pathogenesis [15, 21].

Similar RNA oxidation in the neuronal cytoplasm has also been observed in the brains of patients with Lewy body dementia (LBD) [22], Creutzfeldt-Jakob disease [23] and subacute sclerosing panencephalitis [24]. Further studies have demonstrated that neuronal RNA oxidation is followed by detection of the oxyform of nucleoside in the AD brain [25, 26]. Significantly higher levels of 8-OHG have been identified not only in the brain, but also in the CSF of patients with AD and PD [4, 27] as well as in serum of PD patients [4] compared to age-matched controls, which indicates that 8-OHG might be a useful biomarker of these diseases.

In this study, we analyzed cerebrospinal fluid (CSF) 8-OHdG levels in various neurodegenerative disorders using an ELISA, and investigated factors that might modify these.

Patients and Methods

The study comprised 97 patients with various neurodegenerative disorders which were registered from April 2005 to August 2007 at the Department of Neurology, Georg August University (Goettingen, Germany), and the Department of Neurology, Comenius University (Bratislava, Slovakia), with a final clinical data set and CSF samples available.

Subject consent was obtained in agreement with the Declaration of Helsinki and the local ethics committee, and informed consent from all patients or their caregivers was provided (and is available).

Diagnoses

Alzheimer’s Disease. Eighteen patients with AD were investigated due to suspicion of Creutzfeldt-Jakob disease, because of rapid progression, atypical course or young onset of the disease. The diagnoses were based on NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association) criteria [28]. In the process of further analyses, their data were further stratified according to age at disease onset (younger or older than 65 years), disease duration (less or more than 6 months before the lumbar puncture was performed) and the severity of the dementia.

Dementia with Lewy Bodies. Eighteen patients fulfilled the criteria for probable LBD [29].

Parkinson’s Disease. Forty-eight patients with a diagnosis of PD according to the UK Parkinson’s Disease Society Brain Bank criteria [29, 30] were included in this study.

According to the McKeith criteria [29, 31], PD were patients further divided into a group without dementia (PD) to represent early stages of the disease and a group with dementia (PDD) mirroring advanced stages.

Controls

The control group comprised 13 non-demented patients (MMSE score ranging between 28 and 30 points) who underwent the lumbar puncture to exclude a structural neurological disorder. Their diagnoses were: depression (n = 6), schizophrenia (n = 2), migraine (n = 2), transient global amnesia (n = 1), chronic fatigue syndrome (n = 1) and trigeminal neuralgia (n = 1).

Data Analysis

First, we analyzed the data by the type of diagnosis. Then, we grouped patients without cognitive impairment (PD patients and control group) as the non-dementia group, and the remaining patients (PDD, LBD and AD) as a dementia group.

Considering the potential influence of the severity of dementia on the 8-OHdG levels, we additionally defined the mild/moderate dementia (MMSE score: 20–27) and severe dementia groups.

Age at the time of sampling was categorized into 4 clinically meaningful groups of age, with patients younger than 60 years being the reference group. Regarding the disease duration up to the point when the lumbar puncture was performed, we divided our patients into 3 groups: duration less than 6 months, duration of 6–24 months and duration longer than 24 months after the primary diagnosis was estimated.

Analysis of CSF for 8-OHdG. CSF samples were obtained by lumbar puncture. The routine investigation of the CSF did not reveal any abnormalities with respect to the cell count and proteins. Blood-contaminated samples were excluded from analysis because contamination of this type can lead to abnormal results. CSF samples were centrifuged immediately after lumbar puncture at 2,000 g for 10 min. CSF supernatant was rapidly frozen, and stored at –80°C until the measurement was performed.

All samples were measured concomitantly using the same capture ELISA kit. A commercially available 8-OHdG capture kit was used (8-OHdG Check high sensitive, IBL Transatlantic, Toronto, Canada). The standard measurement range was 0.125–10 ng/ml. Quantification was attained by comparing absorbance intensity to a standard curve.

Statistical Analysis. Descriptive statistics were calculated for every group. Significances were tested by SigmaStat version 3.1 (Systat Software, Point Richmond, Calif., USA) using Student’s t test or the Mann-Whitney rank-sum test; for more than 2 groups, the Kruskal-Wallis test was used. A value of p < 0.05 was considered significant. The correlation between the levels of 8-OHdG and clinical or demographic features were analyzed by Pearson’s correlation coefficient.
Results

Patient Characteristics

Measurements of 8-OHdG proteins by ELISA were performed on 97 patients with various neurodegenerative disorders (AD, LBD, PD, PDD) and healthy controls (46 women, 51 men, ratio 0.90). Clinical and demographic data are displayed in table 1.

8-OHdG Analysis in CSF

The levels of CSF 8-OHdG varied between different neurodegenerative disorders (fig. 1). In the AD group, the median 8-OHdG level was 0.89 ng/ml (0.125–1.85 ng/ml). No significant differences were found with regard to age at the disease onset, disease duration or severity of dementia in the AD group. In the LBD group, a median of 0.81 ng/ml and a mean 0.83 ± 0.25 ng/ml (0.24–1.25 ng/ml) were calculated. The whole PD group showed a tendency towards higher CSF levels (median 0.97 ng/ml and mean 0.98 ± 0.41 ng/ml) as compared to other investigated groups.

In the next step, we evaluated the data by the presence or absence of dementia. In the PDD group, the median 8-OHdG level was 0.95 ng/ml (mean 0.90 ± 0.38 ng/ml, range 0.21–1.73 ng/ml); in PD, the median was calculated to be 0.98 ng/ml (mean 1.0 ± 0.45 ng/ml, range 0.2–2.0 ng/ml). Thus, in both groups, the median levels were very close.

In comparison to these results, we observed the lowest 8-OHdG CSF levels in the control group, where the median reached the value of 0.69 ng/ml (mean 0.71 ± 0.29 ng/ml, range 0.14–1.31 ng/ml). Whereas levels of all PD patients tended to be higher than in controls, only those without dementia were significantly different (p = 0.03).

Although no significant difference was observed between demented and nondemented patients irrespective of the diagnosis, there was a negative trend with dementia severity. The median 8-OHdG CSF level was higher (0.9 ng/ml) in mild/moderate cognitive decline as compared to severe cognitive impairment (0.8 ng/ml), but did not reach a significant level for the whole population studied here. There was a negative correlation (r = –0.59, p = 0.034) between 8-OHdG CSF levels and decline in cognitive functions (evaluated with MMSE) in demented PD patients. However, further statistical analyses regarding the severity of cognitive impairment in PDD group were not significant.

There was no significant difference in any dementia group (AD, LBD, PDD) between mild/moderate dementia in comparison to severe dementia. There was no influence of age at the time of sampling or gender on 8-OHdG CSF levels in all subgroups. Results are displayed in table 2 and figures 1 and 2.

Discussion

Oxidative damage may play an important role in the pathogenesis of several neurodegenerative diseases, and growing evidence points out the involvement of free radicals in mediating neuronal death in these disorders [1–8, 32]. Thus, markers of oxidative damage reflecting the disease and potentially the disease stage and severity may be detectable in body fluids of affected patients. In this work,
we evaluated 8-OHdG levels as a marker of oxidative stress in the CSF of 97 patients to assess the differences between various neurodegenerative disorders. The most strongly elevated 8-OHdG CSF levels were observed in nondemented PD patients, which were significantly higher when compared to the controls.

This is in accordance with the study performed by Abe et al. [27] measuring the concentrations of 8-OHG in the CSF and serum of patients with PD, which were approximately 3-fold higher in PD patients than in controls. Although we could not confirm this fully, our results pointed to the same direction. According to the aforemen-
Table 2. Levels of CSF 8-OHdG in various neurodegenerative diseases and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>8-OHdG levels (range)</th>
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<tbody>
<tr>
<td>Diagnosis</td>
<td></td>
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<tr>
<td>AD</td>
<td>0.89 ± 0.47 (0.125–1.85)</td>
</tr>
<tr>
<td>LBD</td>
<td>0.83 ± 0.25 (0.24–1.25)</td>
</tr>
<tr>
<td>PD</td>
<td>1.0 ± 0.45 (0.2–2.0)</td>
</tr>
<tr>
<td>PDD</td>
<td>0.90 ± 0.38 (0.21–1.7)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.71 ± 0.29 (0.14–1.3)</td>
</tr>
<tr>
<td>Time from diagnosis to lumbar puncture</td>
<td></td>
</tr>
<tr>
<td>&lt;6 months</td>
<td>0.80 ± 0.24 (0.125–2.0)</td>
</tr>
<tr>
<td>6–24 months</td>
<td>0.98 ± 0.40 (0.5–1.9)</td>
</tr>
<tr>
<td>&gt;24 months</td>
<td>0.90 ± 0.36 (0.2–1.8)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>0.95 ± 0.5 (0.125–2.0)</td>
</tr>
<tr>
<td>60–69</td>
<td>0.90 ± 0.3 (0.2–1.6)</td>
</tr>
<tr>
<td>70–79</td>
<td>0.83 ± 0.4 (0.2–1.8)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>0.96 ± 0.2 (0.7–1.3)</td>
</tr>
<tr>
<td>Presence of dementia</td>
<td></td>
</tr>
<tr>
<td>Demented</td>
<td>0.90 ± 0.38 (0.125–1.8)</td>
</tr>
<tr>
<td>Nondemented</td>
<td>0.87 ± 0.42 (0.14–2.0)</td>
</tr>
<tr>
<td>Severity of dementia</td>
<td></td>
</tr>
<tr>
<td>Mild/moderate</td>
<td>0.90 ± 0.4 (0.2–1.9)</td>
</tr>
<tr>
<td>Severe</td>
<td>0.86 ± 0.34 (0.125–1.7)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.95 ± 0.45 (0.125–2.0)</td>
</tr>
<tr>
<td>Women</td>
<td>0.86 ± 0.35 (0.2–2.0)</td>
</tr>
</tbody>
</table>

In the same study, Hoehn-Yahr correlated PD stage with MMSE score; this was not observed for MMSE and disease duration, age at disease onset or age at death. The decrease in MMSE scores in advanced PD stages indicates that the risk of dementia increases with disease progression. With respect to these considerations, one would expect higher levels of oxidative damage products in advanced disease stages, and consequently also in patients with PD and dementia. Our findings are partially in accordance with this hypothesis. Although levels of oxidative damage are higher in all PD patients than controls, the differences were more obvious in nondemented PD patients as compared to the PD group with dementia. However, there was an increase in 8-OHdG CSF levels with lower MMSE score in the PDD group. No significant result was found when the severity of dementia within this group was considered. The trend of 8-OHdG levels to increase with severity of PD dementia suggests the important role of oxidative stress, presumably with other mechanisms, in the disease progression.

Concomitant medication in PD may have a potential influence on neurochemical findings. This has been studied in 66 PD patients using metabolomics. Medication-free patients as well as PD patients receiving dopaminergic therapies were analyzed. The authors demonstrated that the differences between controls and PD subjects were not related to drug effects. Corresponding with our findings, 8-OHdG levels in PD patients were significantly increased as compared to controls [36]. According to these results, we did not stratify our patients according to dopaminergic treatment.

The results obtained in our study are in accordance with a study comparing patients with PD and multiple system atrophy [4]. Significantly higher serum levels, as well as CSF levels of 8-OHdG/8-OHG in PD patients, were observed. Unfortunately, clinically meaningful features, such as presence and severity of dementia, were not taken into account. In our study, 8-OHdG levels were higher in PD patients than in other groups, but no significant difference was observed when the data were stratified by cognitive status.

Several studies have reported that the levels of 8-OHdG/8-OHG in the substantia nigra, caudate nucleus...
and other brain regions were higher in PD than in age-matched controls, which might explain the abnormal findings in the CSF [5, 18]. The main source of increased serum 8-OHG/8-OHG in PD has still not been sufficiently elucidated. Although it is well known that dopaminergic neurons are the most vulnerable cells in PD, various studies have suggested other neuronal cells, skeletal muscle cells [37], lymphocytes [38] and visceral organs [39] are also involved in the disease process, and might therefore be possible sources of increased 8-OHG.

This is the first study to investigate 8-OHdG CSF levels in a group of LBD patients in order to assess its potential role in the differentiation of PDD and LBD as 2 distinct clinical entities; this distinction remains controversial. In our study, there were no significant differences between these groups. Only a limited number of studies have dealt with oxidative damage in both conditions. The extent and distribution of 8-OHG in dopaminergic neurons (cytoplasmic 8-OHG immunoreactivity in substantia nigra) in PD and LBD patients were similar and differed from age-matched controls [40], but the number of positive neurons was significantly less in LBD than in PD patients, suggesting the diminished likelihood of 8-OHG being released into the CSF. This is in accordance with our findings detecting lower 8-OHdG CSF levels in LBD than in PD patients. In another study [22], neurons with marked immunoreaction of 8-hydroxyguanosine were widely distributed in the hippocampal region and temporal neocortex of LBD patients, which is similar to previous findings in the AD brain [41]. In accordance with this, there were no differences in 8-OHdG levels between these subgroups in our study.

The detection of a biomarker in PD represents one of the major challenges for the future because of the importance of the early identification of affected individuals, definition of subgroups and development of neuroprotective treatment strategies. Based on the current results and findings by other authors, we conclude that estimation of 8-OHdG in CSF might be useful in the diagnosing of PD mainly in early stages.

Prospective neuropathological and clinical studies have to follow up this research, in order to establish and verify the potential of 8-OHdG as a marker for diagnosis, prognosis and monitoring of PD.

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