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Oxysterols and Parkinson's disease. Evidence that levels of 24S-hydroxycholesterol in cerebrospinal fluid correlates with the duration of the disease.

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Abstract

Oxysterols are important for cholesterol homeostasis in the brain and may be affected in neurodegenerative diseases. The levels of the brain-derived oxysterol 24S-hydroxycholesterol (24S-OH) have been reported to be markedly reduced in the circulation of patients with Parkinson's disease (PD) (Lee et al., *Antioxid. Redox Signal.* 11 (2009) 407–420). The finding is surprising in view of the fact that other neurodegenerative diseases are associated with relatively modest effects on the circulating levels of 24S-OH. We determined the plasma and cerebrospinal fluid (CSF) levels of 24S-OH and 27-hydroxycholesterol (27-OH) in patients with PD with different disease duration using a highly accurate method based on isotope dilution-mass spectrometry. All the patients had plasma levels of the different oxysterols within the normal range. When analyzing CSF, 10% of the PD patients were found to have levels of 24S-OH above the cut-off level and interestingly there was a significant correlation between levels of 24S-OH in CSF and duration of the disease ($r = 0.40$, $P < 0.05$). The CSF level of 27-OH was found to be above the cut-off level in 10% of the patients, indicating a defect blood–brain barrier function. There was no correlation between levels of 27-OH in CSF and duration of the disease. These data indicates that oxysterol levels in CSF may be of value to follow disease progression.

1. Background

The brain contains about one quarter of whole body cholesterol (for a general reviews, see Ref. [1]). Due to the efficacy of the blood–brain barrier in restricting the entry of cholesterol-rich lipoproteins, the brain meets its substantial requirement for cholesterol by local synthesis.

This synthesis is balanced by efflux of the cholesterol oxidation product, 24S-hydroxycholesterol (24S-OH), which, in contrast to cholesterol itself, is able to pass the blood–brain barrier [2]. Formation of 24S-OH from cholesterol is almost exclusively located to the neuronal cells in the brain. Another side-chain oxidized oxysterol, 27-hydroxycholesterol (27-OH), is taken up by the brain from the circulation [3]. This oxysterol is rapidly metabolized or excreted as such from the brain into cerebrospinal fluid (CSF).

Several studies have highlighted the potential diagnostic utility of the measurement of the concentration of brain derived cholesterol metabolites in plasma or CSF most notably 24S-OH and 27-OH [4], [5], [6], [7] and [8]. It has been shown that a neurodegeneration is associated with increased levels of 24S-OH and 27-OH in CSF, most probably due to a release of the oxysterols from dying neuronal cells. As a consequence of the neurodegeneration and the loss of neuronal cells occurring in connection with later stages of Alzheimer's disease and Huntington's disease, plasma levels of 24S-OH will eventually decrease. It should also be noted that defects in the blood–brain barrier will increase the uptake of 27-OH by the brain and result in increased levels of 27-OH in cerebrospinal fluid [8].

A study has been published according to which plasma levels of 24S-OH are markedly decreased in Parkinson's disease (PD), suggesting measurement of such sterols to be useful in the management of PD patients [9]. The marked changes reported in plasma levels of the oxysterols in PD are surprising in view of the relatively modest changes observed in other neurological diseases [4], [5], [6], [7] and [8]. It is obvious that a confirmative study is needed.

In the present work we have analyzed levels of 24S-OH and 27-OH not only in plasma, but also in CSF, from patients with PD. We could not confirm the study by Lee et al. [9], but found that the levels of 24S-OH in CSF were increased in a relatively large fraction of the patients and that this increase correlated with a longer disease duration.

2. Materials and methods

2.1. Subjects

Two groups of patients with PD were used. None of the patients met the criteria for dementia associated with PD. In the first group, plasma and CSF from 22 Swedish patients with definite PD according to the UK PDSBB criteria was obtained from the Linköping University Hospital, Sweden. The median age of the patients was 66 years (14 males and 8 females).

The CSF samples were obtained by lumbar puncture and collected into polypropylene-tubes and subsequently centrifuged (1300–1800 × g, 4 °C, 10 min). The supernatant was carefully pipetted off and dispensed in 500 μl volumes in polypropylene microtubes before storage at –80 °C. The time interval from collection to freezing was less than 60

min. In the second group, CSF from 8 Danish PD patients were recruited from the Department of Neurology, Bispebjerg Hospital, Copenhagen University Hospital, Denmark. The median age of the patients was 60 years (6 males and 2 females).

CSF was collected in polypropylene tubes, immediately placed in ice water, and subsequently centrifuged ($2000 \times g$, $4\text{ }^{\circ}\text{C}$, 10 min). The supernatant was carefully pipetted off and dispensed into $400\text{-}\mu\text{l}$ aliquots in polypropylene microtubes before storage at $-80\text{ }^{\circ}\text{C}$. The time interval from collection to freezing was less than 90 min. The disease duration of the patients was 3–18 years and all patients were on anti-Parkinsonian medication, including l-DOPA, D2 receptor agonists and MAO B inhibitors.

As controls for the plasma studies, we used a population of patients with a median age of 58 years (19 females and 7 males) with cognitive complaints but without impairment on objective cognitive tasks [7]. A detailed investigation was carried out that excluded presence of Alzheimer's disease. Data from this control group has been presented previously [7].

As controls for the CSF studies we used 35 subjects with a mean age of 58 years (15 males and 20 females) who had been investigated for headache of uncertain background and presenting symptoms without any clinical or laboratory signs. The data from this control group has been presented previously [5].

All the investigations were performed in agreement with the Helsinki declaration and with the permission from the local ethical committee of the hospital.

2.2. Analyses

The analyses of the oxysterols were performed by isotope dilution-mass spectrometry and use of deuterium labelled internal standards as described previously [10].

2.3. Statistics

Descriptive statistics and correlation analyses with Pearson's test followed by t-tests were made with Prism (GraphPAD Prism 5.0).

3. Results

The results of the measurements of oxysterols in plasma are shown in Fig. 1A. The level of 24S-OH in the 22 Swedish PD patients was found to be $61 \pm 4\text{ ng/ml}$. The level of this oxysterol in plasma of a control population of similar age and gender composition was found to be $58 \pm 10\text{ ng/ml}$ [7]. The results are in marked contrast to those obtained by Lee et al. [9] who reported the plasma levels of 24S-OH to be $15 \pm 4\text{ ng/ml}$ in a population of PD patients as compared to $46 \pm 19\text{ ng/ml}$ in the corresponding age- and gender matched control population.

The mean levels of 27-OH in the PD population was found to be $157 \pm 7\text{ ng/ml}$. These levels are lower than those of the present control subjects, $226 \pm 52\text{ ng/ml}$. On the other hand the levels of the PD patients are within the levels regarded as normal [10].

The results of the measurements of oxysterols in CSF are shown in Fig. 1B. The level of 24S-OH in CSF from the 30 Swedish and Danish PD patients was found to be $2.0 \pm 0.2\text{ ng/ml}$ as compared to 1.4 ± 0.5 in the control group. Based on our work with this

methodology and different control populations we have defined a cut-off value of 3 ng/ml for 24S-OH in CSF [6]. This cut-off value corresponds roughly to a mean + 3SD of the levels obtained in control populations when using the present method. According to this cut-off, 3 patients (10%) had pathological levels of 24S-OH in CSF.

The levels of 27-OH in CSF from the PD patients were found to be 1.0 ± 0.3 ng/ml as compared to 0.5 ± 0.1 ng/ml in the control group. Based on previous work with this methodology and several other control populations we have defined a cut-off value of 1.5 ng/ml for 27-OH in CSF [5], [6] and [8]. According to this cut-off value, 3 patients (10%) had increased levels of 27-OH in CSF.

Fig. 2 demonstrates the relation between the levels of 24S-OH (Fig. 2A), 27-OH (Fig. 2B) and the ratio 24S-OH/27-OH (Fig. 2C) in CSF and disease duration in 30 PD patients. Pearson analyses showed that there were significant positive correlations between disease duration and 24S-OH ($r = 0.40$; $P < 0.05$) or 24S-OH/27-OH ($r = 0.33$; $P < 0.05$), but not with 27-OH ($r = 0.25$). No significant correlations were found age and 24S-OH ($r = 0.21$), 27-OH ($r = 0.14$) or 24S-OH/27-OH ($r = 0.07$).

4. Discussion

We could not confirm the surprising finding by Lee et al. [9] that PD patients have a very marked decrease (-67%) in the plasma levels of 24S-OH. The possibility must be considered that the reason for the marked difference between our results and those published by Lee et al., is due to the different methodologies used. In a 2008 methodological paper Lee et al. described their mass spectrometric method in detail [11]. It is noteworthy that the internal standard used in their method is 24-hydroxycholesterol-25,26,26,26,27,27,27-d₇, most likely the racemic mixture of 24R- and 24S-d₇-hydroxycholesterol available from Medical Isotopes. During fragmentation of the trimethylsilyl ether of 24-hydroxycholesterol in the mass spectrometer, the most prominent ion, m/z 413, is formed by loss of the end of the steroid side-chain. Due to the position of the deuterium label, the intense fragment generated from the above internal standard will have the same mass as the corresponding ion generated from unlabeled 24-hydroxycholesterol. We use racemic d₄-24-hydroxycholesterol with the deuterium in positions 23,23,24,25 as internal standard. The deuterium atom at the 25-position will be lost during the fragmentation, leaving a fragment containing 3 atoms of deuterium. The internal standard will thus generate a fragment m/z 416 in this case. This means that the intense fragment at m/z 413 can be used for quantitation in our method but not in the method used by Lee et al. Instead they use the fragment m/z 456 (M-90) for tracing the unlabeled steroid and the fragment at m/z 463 for tracing the d₇-labelled internal standard. The fact that the intensity of the fragment m/z 456 is considerably lower than that of m/z 413 is a disadvantage. In addition to the lower sensitivity of the ion m/z 456 the specificity is lower due to the fact that nearly all monohydroxylated cholesterol metabolites have the same molecular masses for their trimethylsilylated ethers.

Another potentially important difference between our method and the one used by Lee is the use of different solvents and lower amounts of these solvents in connection with the elution of the cholesterol oxidation products from the SPE columns. This may cause a lower recovery of the oxysterols. In a recovery experiment with plasma spiked with 24-

hydroxycholesterol (not known if 24S-hydroxycholesterol or the racemate is used) the spiked concentrations at the x-axis was not linear and the curve should become nearly exponential at the 50 ng/ml level [11].

In a more recent publication [12] Lee et al. reported extremely low levels of 24S-hydroxycholesterol in plasma, 1.4–3.90 ng/ml in patients with Parkinson's disease treated with L-DOPA. These levels were not significantly different from those of the controls. Since the same method was used in that study as in the previous studies by the same authors [9] and [11] it seems likely that there are problems with the method by Lee et al. and that this is the explanation for the difference in our results and those reported by Lee et al.

In addition to the data presented here, we have measured plasma oxysterols in a number of other PD patients but always found normal levels of 24S-OH (unpublished observation). It is evident that plasma levels of 24S-OH cannot be used as a diagnostic tool in connection with PD as suggested by the results of Lee et al. [9].

The circulating levels of 27-OH in our PD patients were lower than those of the present control population but within the normal levels defined for 27-OH [10]. It may be mentioned that low levels of 27-OH in the circulation has been reported also in other neurodegenerative diseases such as Huntington's disease and multiple sclerosis [8]. Plasma levels of 27-OH are affected by cholesterol levels and gender. Our group [10] as well as other groups have observed that males have about 20% higher levels than females. In contrast there are no significant gender differences in plasma levels of 24S-OH. It is noteworthy that Lee et al. failed to demonstrate a gender difference in circulating levels of 27-OH with their method.

Evidence has been presented that 24S-OH in CSF is a sensitive marker for early neurodegeneration [6]. The levels of 24S-OH in CSF were above the cut-off level only in 10% of the patients. However, an interesting observation was that there was a significant correlation between CSF levels of 24S-OH and disease duration of the PD patients.

The levels of 27-OH in CSF were also above the cut-off value in 10% of the PD patients. Such increase may be explained by two mechanisms. A neurodegeneration leads to reduced number of neuronal cells and thus decreased capacity to metabolize 27-OH by the neuronal specific enzyme CP7B1. A defect blood–brain barrier leads to increased flux of 27-OH into the brain with increased levels in CSF [8]. There are previous reports that the blood–brain barrier may be defect in a high proportion of PD patients and the possibility has been discussed that this may be of importance for the pathogenesis of the disease.

In summary we could not confirm that levels of the side-chain oxidized oxysterols in plasma are of diagnostic value in Parkinson's disease. However, our data indicate that the levels of these oxysterols, particularly 24-OH, in CSF may reflect neurodegenerative changes occurring during disease progression. More studies with more patients are needed, however, to confirm and establish this.

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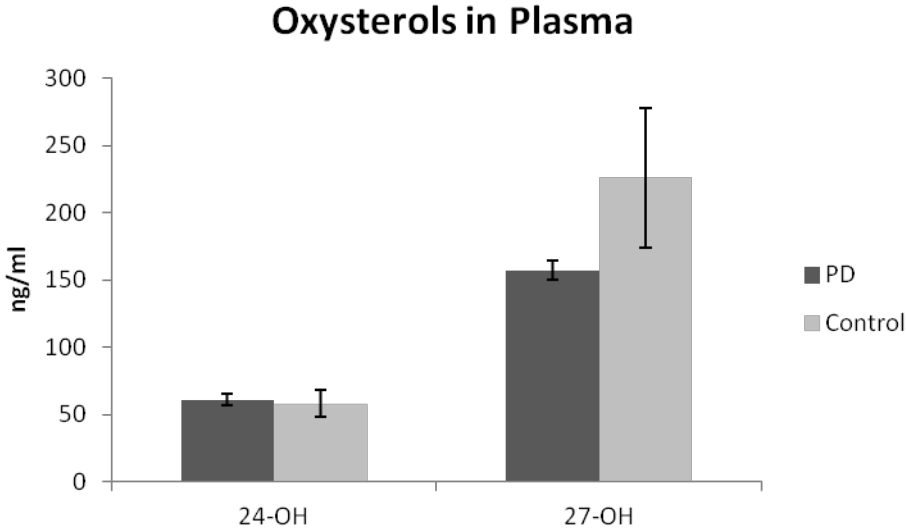
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Figure Legends

Fig. 1. (A) Levels of 24S-OH and 27-OH in the circulation of PD patients (n = 22) and controls. The levels for the control population have been published previously [8]. (B) Levels of 24S-OH and 27-OH in CSF from 30 PD patients and controls. The levels for the control population have been published previously [6].

Fig. 2. Relation between levels of 24S-OH (A), 27-OH (B) and the ratio 24S-OH/27-OH (C) in CSF and duration (in years) of the disease in 30 PD patients.

Figure 1



B

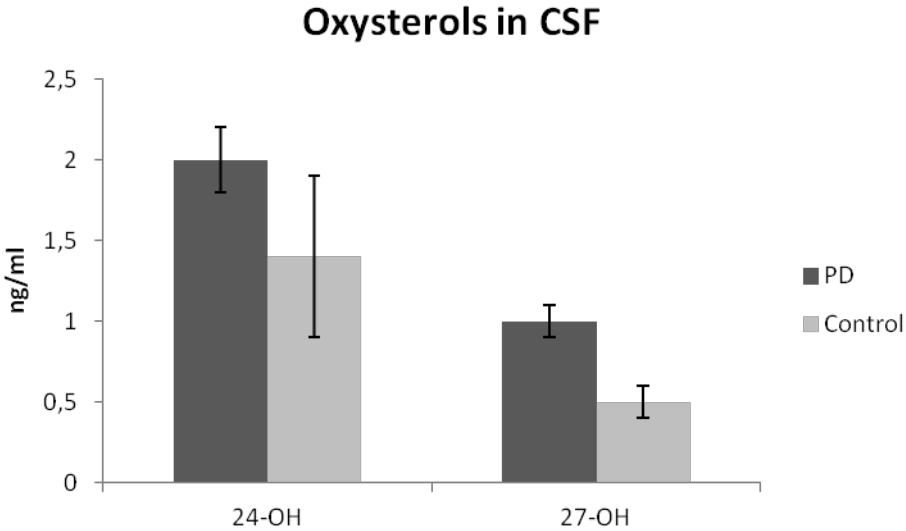
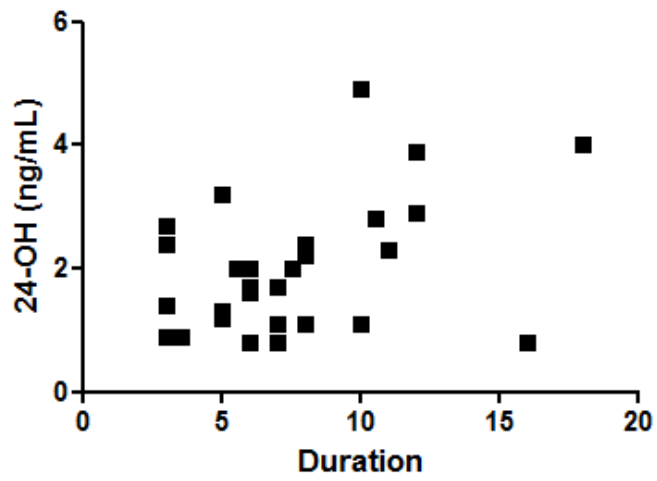
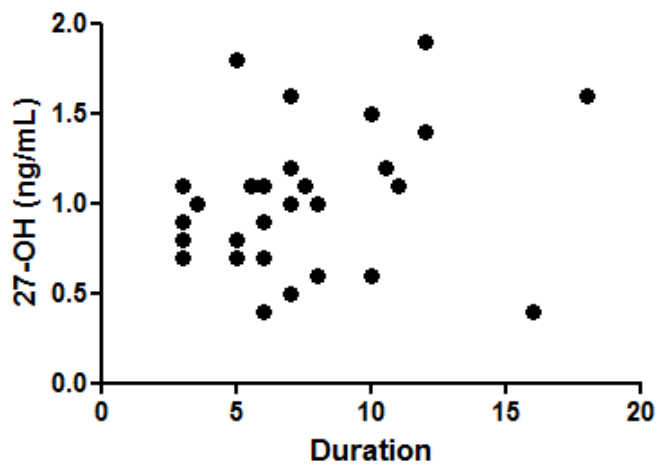


Figure 2

A



B



C

