Neuroscience Letters 555 (2013) 102–105

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

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

Ingemar Björkhem[a,*], Anita Lövgren-Sandblom[a], Valerio Leoni[c], Steve Meaney[d], Lovisa Brodin[b], Lisette Salveson[e], Kristian Winge[e], Sven Pålhagen[b], Per Svenningsson[b]

[a] Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden
[b] Department of Neuroscience, Karolinska Institute, Stockholm, Sweden
[c] IRCCS Institute of Neurology "Carlo Besta", Milano, Italy
[d] School of Biological Sciences, Faculty of Science, Dublin Institute of Technology, Dublin, Ireland
[e] Department of Neurology, Bispebjerg University Hospital, Copenhagen, Denmark

HIGHLIGHTS

- 24S-hydroxycholesterol in plasma is not a suitable biomarker for Parkinson's disease.
- Levels of 24S-hydroxycholesterol in CSF are correlated to duration of the disease.
- Levels of 27-hydroxycholesterol are normal in plasma but increased in CSF in a subfraction of patients.

ARTICLE INFO

Article history:
Received 20 July 2013
Received in revised form 27 August 2013
Accepted 1 September 2013

Keywords:
Biomarkers
Oxysterols
24S-hydroxycholesterol
27-Hydroxycholesterol

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 [4–8]. It has been shown that a neurodegeneration is

* Corresponding author. Tel.: +46 8 58581235; fax: +46 8 58581260.
E-mail address: ingemar.bjorkhem@karolinska.se (I. Björkhem).

0304-3940/$ – see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.neulet.2013.09.003

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 [4–8]. 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 × *g*, 4 °C, 10 min). The supernatant was carefully pipetted off and dispensed into 400-μl aliquots in polypropylene microtubes before storage at −80 °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.

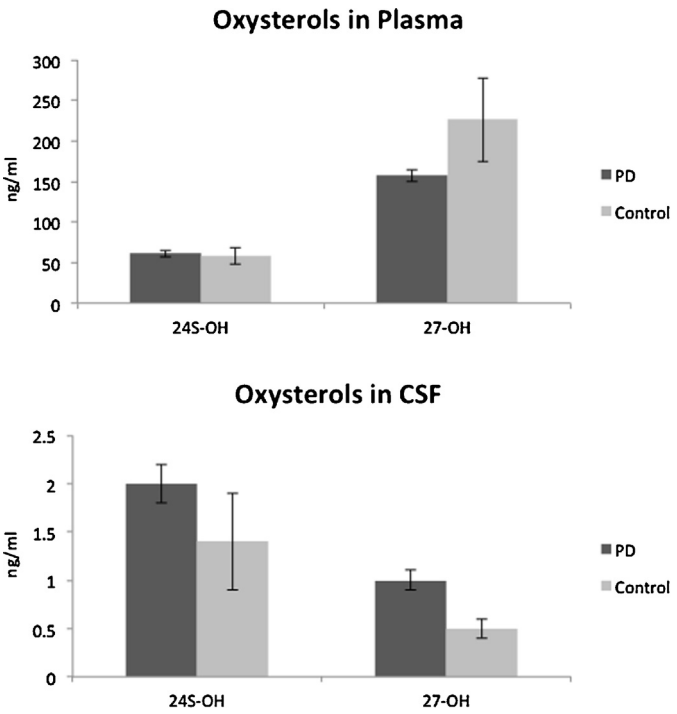

Fig. 1. (A) Levels of 24S-OH and 27-OH in the circulation of PD patients ($n = 22$) and controls. The levels for the control population have been published previously [8]. (B) Levels of 24S-OH and 27-OH in CSF from 30 PD patients and controls. The levels for the control population have been published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

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 ± 4 ng/ml. The level of this oxysterol in plasma of a control population of similar age and gender composition was found to be 58 ± 10 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 ± 4 ng/ml in a population of PD patients as compared to 46 ± 19 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 ± 7 ng/ml. These levels are lower than those of the present control subjects, 226 ± 52 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 ± 0.2 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