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Reduced cerebrospinal fluid concentrations of oxysterols in response to natalizumab treatment of relapsing remitting multiple sclerosis

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ABSTRACT

Background: Natalizumab therapy reduces inflammation and degeneration of the CNS in relapsing-remitting multiple sclerosis (RRMS). In cerebrospinal fluid (CSF) the concentration of 24S-hydroxycholesterol (24OHC) reflect neurodegeneration, whereas 27-hydroxycholesterol (27OHC) is dependent on the integrity of the blood-brain barrier (BBB).
Objective: To measure the impact from natalizumab treatment on 24OHC and 27OHC concentrations in serum and CSF of RRMS.
Methods: In serum and CSF obtained from 31 patients before and following 12 months of natalizumab treatment, 24OHC and 27OHC were analyzed by isotope-dilution mass spectrometry.
Results: Natalizumab treatment reduced CSF-24OHC concentrations ($p = 0.002$), CSF-27OHC concentrations ($p = 0.01$) and serum-24OHC concentrations ($p = 0.029$). There was no significant effect of the treatment on serum-27OHC concentrations. Serum concentrations of 24OHC correlated with Symbol Digit Modalities Test scores before ($r = 0.5$, $p = 0.007$) and after natalizumab treatment ($r = 0.403$, $p = 0.033$).
Conclusions: We showed for the first time that natalizumab treatment of RRMS reduced the concentrations of 24- and 27OHC in CSF, indicating reduced neurodegeneration and improved integrity of the BBB, respectively. Our results imply a role for serum 24OHC as a biomarker of cognition (visuo-spatial ability and processing speed) in RRMS.

1. Introduction

24S-hydroxycholesterol (24OHC, cerebrosterol) is a brain-specific cholesterol hydroxylation product that passes freely over the blood-brain barrier [1]. Almost all 24OHC in blood circulation originates from the brain [1–3] and in healthy individuals the concentrations seem constant throughout adult life [4]. It has been shown in several studies that serum concentrations of 24OHC reflect CNS cholesterol turnover [2,3,5] and is suggested as a potential biomarker for neurodegeneration [6,7] reflecting the amount of metabolically active neurons. Less than 1% of 24OHC is excreted into the cerebrospinal fluid (CSF) [6], but this fraction may increase as a consequence of neuronal damage [8]. In contrast, almost all cells of the body can synthesize 27-hydroxycholesterol (27OHC), which is another side-chain oxidized oxysterol. The level of this oxysterol in CSF correlate with the corresponding levels in the circulation [9] and the flux into the brain is to some extent dependent upon the integrity of the blood-brain barrier (BBB) [9]. Because the metabolism of 27OHC in the brain is dependent upon CYP7B1, an enzyme located in neuronal cells, neuronal loss may lead to increased concentrations of 27OHC in CSF [10].

In active lesions of multiple sclerosis (MS) the neuronal tissue integrity is damaged and axonal loss occurs [11]. This is the culprit of cerebral atrophy and development of neurological disability in MS. It has been suggested that increased serum concentrations of 24OHC in MS patients may reflect enhanced neuronal damage [12] and they also correlate to cerebral atrophy in relapsing-remitting (RR) MS [13]. In neurodegenerative diseases such as Alzheimer disease, Huntington disease and also MS, decreased concentrations of 24OHC was found in the peripheral circulation, indicating reduced flux from the brain and/or increased neuronal loss [4,12,14–16].

Neurofilament light (NFL) is a cytoskeletal protein of myelinated axons and is regarded as a CSF biomarker of axonal damage. Increased

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CSF-NFL concentrations are found during all stages of MS and peak during acute relapses or during the appearance of contrast enhancing lesions on MRI [17,18]. Treatment of MS with natalizumab (NZ), a monoclonal antibody that inhibit leucocyte migration over the BBB, effectively reduce cerebral inflammation [19,20]. NZ therapy of RRMS also reduce NFL concentrations, comparable to concentrations, found in healthy controls (HCs) [21]. Thus, effective immunomodulation of MS seems also to reduce the rate of neurodegeneration.

In the present study we followed both serum and CSF concentrations of 24OHC and 27OHC in parallel with CSF concentrations of NFL before and after NZ treatment of RRMS patients. The aim was to explore oxysterols as potential biomarkers of the immunopathogenesis of MS and their usefulness as markers of disease activity, disease progression and therapeutic efficacy.

2. Materials and methods

2.1. Patients and healthy controls

The study was approved by the regional ethical board of the University of Gothenburg, Sweden. Informed consent was obtained from patients and HCs upon their recruitment to the study. The study cohort consisted of 31 RRMS patients and 16 HCs. Patients had been diagnosed with MS according to the revised McDonald criteria [22]. They were recruited prospectively at the MS Center, Department of Neurology, Sahlgrenska University Hospital, Gothenburg, Sweden, and constituted a sub-group of a larger population that had been described previously [21]. Disease duration was estimated from onset of the first demyelinating symptoms. Patients were treated with NZ 300 mg intravenously once monthly. Three patients had no previous treatment, 8 were previously treated with mitoxantrone and 20 were previously treated with interferon beta or glatiramer acetate. Patients treated with mitoxantrone terminated their treatment on average 6 months (range 3–12) prior to natalizumab treatment. Ten patients had relapse within 3 months before assessment and 21 were in remission. Of those ten patients who had relapse, 8 had relapse prior and 2 had relapse during treatment with NZ.

2.2. Clinical assessment and specimen sampling

Neurological disability was scored using the Expanded Disability Status Scale (EDSS) [23] and cognitive function (visuo-spatial ability and processing speed) was tested with the Symbol Digit Modalities Test (SDMT) [24–27]. None of the HCs had a history of neurological disease and all had normal clinical neurological examination. SDMT was not tested in HCs. Serum and CSF were sampled once in HCs and at baseline and after 12 months of NZ treatment in patients. Lumbar puncture was done according to the procedures recommended in the consensus protocol of the BioMS-EU network for CSF biomarker research in MS [28]. CSF was transported on ice and the first 12 mL of CSF was carefully mixed; after centrifugation, fractions were snap-frozen within 2 h in 0.5 mL aliquots and stored at −80 °C until analysis.

2.3. Determination of albumin ratio and serum cholesterol

Albumin concentration in serum and CSF was measured by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). CSF/serum albumin ratio, as a function of the permeability of the BBB, was calculated as CSF albumin (mg/L) divided by serum albumin (g/L). Serum (S) cholesterol concentration was determined using an enzymatic colorimetric method on the c501 module of the Roche 6000 analyzer according to instructions from the manufacturer (Roche, Penzberg, Germany).

2.4. NFL enzyme-linked immunosorbent assay

CSF NFL protein was measured with a sensitive sandwich ELISA method (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden) by board-certified laboratory technicians at the Clinical Neurochemistry Laboratory, the Sahlgrenska University Hospital, according to protocols approved by the Swedish Board for Accreditation and Conformity Assessment. The lower limit of quantification of the assay was 31 ng/L. Intra- and inter-assay coefficients of variation were below 10%.

2.5. 24OHC and 27OHC spectrometry

24OHC and 27OHC in serum and CSF were analyzed by isotope-dilution mass spectrometry using deuterium-labeled internal standards, as described elsewhere [29]. In brief, serum and CSF samples were subjected to saponification to hydrolyze oxysterol esters. The hydrolysis was performed at 22 °C for 2 h with concentration 0.35 M KOH. In order to prevent cholesterol autoxidation during sample preparation and handling, cholesterol was separated from oxysterols by solid-phase extraction using silica columns. A deuterium-labeled internal standard were added for both oxysterols analyzed. 27OHC and 24OHC are the most abundant cholesterol oxidation products (mean serum values 154 and 64 ng/ml respectively in a healthy population).

2.6. Statistics

Because of the small sample size and non-normal distribution of serum 27OHC (Shapiro-Wilk test, $p < 0.05$), non-parametric statistics was used. Mann–Whitney U-test were used to investigate group differences and Wilcoxon signed rank sum test were used for analysis of matched pair data, i.e. before and after NZ treatment. Correlation coefficients were calculated using Pearson and Spearman two-tailed correlation test. Multiple regression analysis included the following variables: age, gender, disease duration, EDSS, SDMT and relapse within 3 months before assessment. Statistical calculations were performed in SPSS Statistics 22 software and in Microsoft Office Excel 2013.

3. Results

Clinical characteristic and demographic features of patients and HC are presented in Table 1. NZ treatment did not influence S-cholesterol concentration. Mean S-cholesterol was 4.68 (SD = 1.0) mmol/l prior

Table 1
Clinical characteristic and demographic features.

	All patients (n = 31)	Patients in remission (n = 21)	Patients with relapse (n = 10)	HC (n = 16)
Gender male/female, no	11/20	8/13	3/7	11/5
Mean age, years (range)	36 (13–60)	35 (13–60)	39 (26–53)	41 (27–53)
Mean disease duration, years (range)	8.4 (0.5–26)	7.9 (2–26)	9.5 (0.5–21)	NA
Median EDSS before treatment (range)	3.5 (0–6.5)	3.0 (0–6.5)	5.0 (2.5–6.5)	NA
Median EDSS after treatment (range)	3.5 (0–6.5)	2.5 (0–6.5)	4.5 (0–6.5)	NA

HC: healthy controls.