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The soluble transcobalamin receptor (sCD320) is present in cerebrospinal fluid and correlates to dementia-related biomarkers tau proteins and amyloid-beta

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Abstract

Background. Cellular uptake of vitamin B12 (B12) demands binding of the vitamin to transcobalamin (TC) and recognition of TC-B12 (holoTC) by the receptor CD320. Recently, we identified a soluble form of CD320 (sCD320) in human plasma. Here we present data on the occurrence of this soluble receptor in cerebrospinal fluid (CSF) and show its correlations to dementia-related biomarkers tau proteins and amyloid-beta.

Methods. We collected 223 cerebrospinal fluid samples and corresponding plasma samples (n = 46). We measured CSF and plasma sCD320, holoTC and total TC employing in-house ELISA methods and CSF phospho-tau (181P) (p-tau), total tau (t-tau) and amyloid-beta 1-42 (Aβ) (n = 177) employing commercial ELISA kits (Innogenetics Company). Size exclusion chromatography was performed on a Superdex 200 column.

Results. The median sCD320 concentration in CSF (14 pmol/L) is around five times lower than in plasma (72 pmol/L). No correlation was observed between plasma and CSF sCD320 levels (n = 46), while the behavior upon size exclusion chromatography was the same. In CSF, sCD320 correlates to holoTC and total TC (Spearman’s correlation (Rs) = 0.325, 0.232 (n = 218, 217) respectively, p < 0.01). Interestingly, sCD320 correlates to p-tau and t-tau (Rs = 0.599, 0.569 (n = 173, 176) respectively, p < 0.001) and to Aβ (Rs = 0.265, p < 0.001 (n = 177)).

Conclusion. We document for the first time the occurrence of sCD320 in human CSF. We report that the concentration of sCD320 correlates to the dementia-related biomarkers p-tau, t-tau and Aβ.

Key Words: CD320, cobalamin, vitamin B12, phosphorylated tau, amyloid beta-protein (1-42)

Introduction

Vitamin B12 (B12) is essential for nervous system development and functions [1,2] and its deficiency is associated with cognitive impairment and neurodegenerative disease [3,4]. The transport of B12 from its food source to reach the nervous system cells is mediated by a complex set of carrier proteins, receptors and transporters [5]. Cellular uptake demands binding of the vitamin to transcobalamin (TC) and recognition of TC-B12 (holoTC) by the receptor CD320 [6]. We recently developed an ELISA method for soluble CD320 (sCD320) measurement and demonstrated its presence in human plasma and urine [7–9]. Like its precursor protein, sCD320 is heavily glycosylated and behaves as a 58-kDa molecule upon SDS-PAGE [7]. The soluble form retains the ability to bind the CD320 ligand, holoTC, and the plasma concentration of holoTC and its soluble receptor show a positive correlation [8]. Recent studies have shown that mice not expressing CD320 have a disturbed B12 metabolism in the brain [10]. This strongly supports the functional presence of CD320 in the normal brain, and promoted our search for the presence of the soluble receptor in human CSF.

Dementia is a syndrome of progressive, global decline in cognition that is severe enough to degrade the individuals’ wellbeing and social function [11]. A combination between a decreased amyloid-beta 1-42 (Aβ) and an increased total tau (t-tau) and phospho-tau (181P) (p-tau) is used for dementia diagnosis and treatment follow-up. An increase in CSF t-tau is also a marker for cell injury and neuronal death [12–14].
In this study, we present data to show the presence of sCD320 in CSF and report its correlation to dementia and neurological injury biomarkers, while no correlation was observed between plasma and CSF levels of sCD320.

**Materials and Methods**

**Samples**

We used anonymized human CSF and plasma samples destined for discard after testing in the routine clinical laboratory at Aarhus University Hospital. Personal information was de-identified from the samples prior to analysis. We analyzed 177 CSF samples (set 1) to investigate the presence of sCD320 in CSF and its correlation to both B12 and dementia-related biomarkers. We extended this study by including 46 paired CSF-plasma samples (set 2) to investigate if sCD320 in CSF and plasma are correlated and to investigate the correlation of CSF-sCD320 to CSF-total protein, albumin, IgG or CSF/plasma albumin ratio (dementia-related biomarkers are not measured in the paired samples). Samples were collected during the 5-month period starting February 2013. The samples were frozen at $-20^\circ$C after receipt from the routine clinical laboratory and thawed immediately prior to analysis. The routine lab (from which the samples were taken) centrifuges the thawed immediately prior to analysis. The routine lab (from which the samples were taken) centrifuges the samples within 4 hours of collection (at 2000 g/L). The samples were frozen at $20^\circ$C (within 48 hours after centrifugation). The samples could not be traced back to the patient. Therefore, according to Danish law our study was exempt from ethical board review.

**Biochemical analysis**

We measured sCD320 employing an in-house ELISA essentially as previously described [7,8]. In brief we used an in-house catching monoclonal antibody against human CD320 (a kind gift from E. Quadros) [15] insolubilized on ELISA plates, 1 μg per well (blocked thereafter with 200 μL of 2 mol/L ethanolamine, pH 8–9). The detecting antibody (diluted to dispense 4 ng per well) was a biotinylated polyclonal antibody against human CD320 (R&D Systems, Minneapolis, MN, USA, catalog #: BAF1557, www.rndsystems.com). The detecting system was based on horseradish peroxidase-avidin (Dako, Glostrup, Denmark, catalog #: P0364, www.dako.com) and TMB ONE Ready-to-use Substrate (KEM EN TEC, Taastrup, Denmark, catalog #:4380A, www.kem-en-tec-nordic.com), Calibrators in concentrations from 1.5 to 104 pmol/L was prepared from recombinant sCD320 (R&D Systems, Denmark, catalog #: 1557-CD-050, www.rndsystems.com). For each assay a plate was washed three times with washing buffer (10 mmol/L sodium phosphate, 145 mmol/L NaCl, 1 g/L bovine albumin and 1 g/L Tween 20 pH 7.4) and 100 μL calibrator, control or sample were added to individual wells. After incubation overnight at $4^\circ$C, the plates were washed three times with washing buffer and incubated overnight at $4^\circ$C with 100 μL detecting antibody. After an additional three fold washing, 100 μL of horseradish peroxidase-avidin diluted 1:2000 was added to each well. The plates were incubated for 30 min at room temperature, and washed three times, before adding 100 μL TMB. The color reaction was stopped after 12 min by adding 100 μL 1 mol/L phosphoric acid and measured at 450 nm with correction for the absorbance at 620 nm. The calibration curve was constructed based on a cubic spline function. CSF samples were run undiluted, while plasma samples were diluted 1:3 with 0.1 mol/L sodium phosphate buffer containing 1 g/L bovine albumin (Sigma Aldrich, St. Louis, MO, USA, catalog #: A7030, www.sigmaaldrich.com), pH 8.0.

The inter-assay imprecision for control samples with a mean concentration of 17 pmol/L (25 runs over a period of 4 months) was 8.0% and the intra-assay imprecision was 4.3% [7]. The lower limit of detection is $\approx 1.5$ pmol/L (defined as the concentration corresponding to a signal 5 SD above the mean of the sCD320-free calibrator) [7]. The dynamic range is from 3–52 pmol/L (range over which there is a linear relationship between the sCD320 concentration and the absorbance reading).

Total TC was measured by an in-house sandwich ELISA (inter-assay imprecision of 4–6% and an intra-assay imprecision of 3%) [16]. HoloTC was measured by the TC-ELISA after removal of the apoTC with B12-coated beads (inter-assay imprecision of 8% and an intra-assay imprecision of 4%) [17,18]. P-tau, t-tau and amyloid-beta 1-42 were measured employing commercial ELISA kits (Innogenetics Company, Belgium) with intra- and inter-assay imprecision of $(<5, <10)$ (3.6, 3.9) and (3.8, 7.7) % respectively [19,20]. Size exclusion chromatography was performed on a Superdex 200 HR 10/30 column (GE Healthcare Europe, Freiburg, Germany) attached to a Dionex ICS-3000 chromatography system (Dionex Corporation, Sunnyvale, CA, USA), as detailed previously [8]. Total CSF-protein, albumin and IgG and plasma albumin were measured on the automatic platform Cobas 6000 (Roch, Tokyo, Japan, http://www.roche.com/) with intra- and inter-assay imprecision of (1.4, 2.4) (1.9, 2.6) and (1.6, 2.5) % respectively.

**Statistical analysis**

B12-related parameters and dementia biomarkers did not follow normal distribution (using Kolmogorov-Smirnov normality tests). Thus, non-parametric
statistical tests were used and measurements were reported as medians with 2.5–97.5th percentiles. Spearman’s rank test was used to investigate the correlations between study variables. Mann-Whitney U test was applied for testing the difference between median levels. Outliers are not excluded as we used statistical methods that minimize the influence of outliers. Since no statistical difference was observed for sCD320 amongst samples collected as set 1 and 2 the data were pooled where appropriate. Statistical analyses were performed using SPSS statistical computer software (version 20, IBM Inc.).

Results

We identified sCD320 in CSF and report its concentration to be a median (2.5–97.5th percentiles) of 14 (7–27) pmol/L, n = 223. For comparison, the concentration of sCD320 in the paired plasma and CSF samples were 72 (48–375) and 13 (8–28) pmol/L respectively, n = 46. In accord with our previous study [21] we measured holo and total TC concentrations in CSF that were two to three fold lower than in plasma, respectively (Table I).

We explored the gel filtration profile of CSF sCD320. ELISA reactivity for sCD320 eluted as a sharp peak with a stokes radius ≈ 50 Å (Figure 1).

In CSF, sCD320 correlates to holoTC (0.325, \( p < 0.01 \), n = 218) and total TC (0.232, \( p < 0.01 \), n = 217). This correlation pattern also exists in plasma [8]. HoloTC and total TC present in plasma (n = 45) correlated with those observed in CSF with Spearman’s correlation coefficients of 0.338, \( p < 0.05 \) and 0.634, \( p < 0.01 \) respectively. This is in agreement with previous findings [21,22]. In contrast we observed no correlation between sCD320 in plasma and CSF (0.032, \( p = 0.834 \), n = 46).

We explored the relation between CSF sCD320 and the dementia biomarkers (p-tau, t-tau and Aβ). CSF sCD320 showed a strong Spearman’s correlation to p-tau (0.599, \( p < 0.001 \), n = 173) and t-tau (0.569, \( p < 0.001 \), n = 176) but a weaker correlation to Aβ (0.265, \( p < 0.001 \), n = 177) as depicted in Figure 2.

No correlation was found between sCD320 and total protein, albumin, albumin CSF/plasma ratio or IgG in CSF (\( p \)-value = 0.293, 0.233, 0.18 and 0.561 respectively, n = 46).

Patients samples with biomarkers suggesting Alzheimer’s dementia (p-tau > 60, t-tau > 450 and Aβ < 500 ng/L, n = 18) showed a significantly (\( p = 0.002 \)) higher concentration of sCD320 (median = 17 pmol/L) than those with normal concentrations for the three biomarkers (median = 13 pmol/L, n = 70) (Figure 2D).

Discussion

This study is the first to report the occurrence of the soluble transcobalamin receptor, sCD320, in CSF. On top of its mere presence, our study shows that...
CSF-sCD320 correlates to dementia-related biomarkers in CSF (p-tau, t-tau and Aβ).

Four observations suggest that CSF sCD320 is derived from local production rather than transferred from the circulation. First, the CSF/plasma concentration ratio of sCD320 is high as compared to other plasma-derived CSF proteins such as albumin [23]. Second it has been shown that neural tissues express CD320 mRNA in amounts comparable to other tissues [24] and that CD320 knockout mice have an impaired neural uptake of the vitamin [10]. Third, we found no correlation between CSF and plasma sCD320. Fourth, the absence of correlation between sCD320 and the CSF/plasma albumin ratio (an indicator for the blood-brain barrier function [25]).

Interestingly the molecular behavior of sCD320 in CSF (in term of size and shape, based on gel filtration profiles) is comparable to that of plasma and urinary sCD320 [8], thus suggesting that sCD320 is a uniform molecule across compartments.

Three dementia-related biomarkers are currently used in the routine clinical setting. A high level of t-tau and p-tau combined with a low level of Aβ (based on cutoff concentrations of >450, >60 and <500 ng/L respectively) supports a diagnosis of Alzheimer’s dementia. We observed a strong correlation between tau proteins and sCD320, while the correlation between Aβ and sCD320 was weaker, though still significant. T-tau is a marker of axonal degeneration and therefore not specific for dementia [13]. Axonal degeneration may enhance sCD320 shedding, resulting in higher CSF levels and t-tau-sCD320 correlation. Alternatively, as CSF t-tau is primarily originated from neurons, the correlation to sCD320 may indicate that both are released in accord from decaying neurons. The current concept that both B12 deficiency and dementia cause neuronal and brain damage [22,26,27] is in accord with these explanations.

In conclusion, we report the presence of sCD320 in CSF and its relation to its ligand holotC. Also we show for the first time a correlation between the soluble transcobalamin receptor and tau proteins. Our results warrants further studies in order to explore the possible use of sCD320 as a biomarker for dementia or neuronal degeneration.

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