Different CSF β -amyloid processing in Alzheimer's and Creutzfeldt–Jakob disease

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Abstract Decreased levels of β -amyloid (A β) 1–42 in cerebrospinal fluid (CSF) are characteristic for Alzheimer's disease (AD) and are also evident in Creutzfeldt–Jakob disease (CJD). A β plaques are thought to be responsible for this decrease in AD patients, whereas such A β plaques are rarely seen in CJD. To investigate the A β pattern in brain and CSF of neuropathologically confirmed CJD and AD patients we used an electrophoretic method to investigate A β peptide fractions which are not accessible to ELISA and immunohistochemistry. We analyzed A β peptides in the CSF of autopsy-confirmed CJD and AD patients and the corresponding brain homogenates using a quantitative

urea-based A β electrophoresis immunoblot (A β -SDS-PAGE/immunoblot). The CSF A β 1–42 decrease correlated with the brain A β load in AD, but not in CJD. There was no difference in the soluble fractions of brain homogenate in AD and CJD. We therefore conclude that different mechanisms in AD and CJD are responsible for the A β 1–42 decrease in the CSF.

Keywords β -amyloid · β -amyloid plaques · Creutzfeldt–Jakob disease · Alzheimer's disease · Cerebrospinal fluid

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Introduction

In Alzheimer's disease (AD) extracellular amyloid beta (A β) peptides form a major component of senile A β -plaques deposited in the brain (Glennner et al. 1984; Hardy and Selkoe 2002). A β peptides are generated from transmembrane β -amyloid precursor protein (APP) by proteolytic enzymes (Citron et al. 1996; Haass et al. 1993). A decrease of A β 1–42 intracellular amyloid peptides cerebrospinal fluid (CSF) is characteristic for AD and may reflect A β plaques in the brain acting as an A β 1–42 “sink”, hindering the transport of soluble A β 1–42 between the brain and CSF (Fagan et al. 2006; Motter et al. 1995). CSF A β 1–42 is known to also be reduced in Creutzfeldt–Jakob disease (CJD), although senile A β plaques are rarely present (Hainfellner et al. 1998; Otto et al. 2000; Wiltfang et al. 2003).

A quantitative urea-based A β -sodium-dodecylsulphate-polyacrylamide-gel-electrophoresis with western immunoblot (A β -SDS-PAGE/immunoblot) recently revealed the regular abundance of several C- and amino terminal-truncated A β peptides in CSF and brain, which displayed disease-specific variations in its absolute and relative

quantities in the CSF of patients with AD and CJD (Wiltfang et al. 2002, 2003).

Our aims are

1. to reconfirm in an independent cohort the different CSF A β pattern in neuropathologically confirmed AD and CJD, which we previously only described based on clinical diagnoses (Wiltfang et al. 2003);
2. to further analyze possible correlations between the A β pattern in CSF and the corresponding frozen brain tissue; and
3. to determine whether other A β peptide fractions which are not accessible to ELISA and immunohistochemistry may explain the decrease of CSF A β 1-42 in CJD using the A β -SDS-PAGE/immunoblot.

We analyzed CSF and the concomitant brain samples with A β -SDS-PAGE/immunoblot from neuropathologically verified CJD and AD patients and controls (Wiltfang et al. 2002, 2003).

Patients and methods

We investigated a total of 42 CSF and 34 brain samples from patients of two diagnostic groups (AD and CJD) and two control groups according to their autopsy-confirmed (CGp) or clinical (CGc) diagnoses (demographics see Table 1).

Diagnostic groups

Subjects with Creutzfeldt–Jakob disease

In detail, we investigated CSF and tissue of the frontal cortex in four men and five women with autopsy-confirmed sporadic CJD. All patients suffered from sporadic CJD and were seen during lifetime and evaluated clinically at the

national surveillance unit for transmissible spongiform encephalopathies located in Goettingen, Germany according to the established criteria (WHO 1998). Mean age at the time of LP was 61 ± 16 (mean $\pm$ standard deviation). Mean number of days in between lumbar puncture (LP) and death of those patients where frontal cortex was available was 222 ± 265 . Mean disease duration (from the beginning of the first symptoms until death) was 10.5 ± 9.3 months.

Subjects with Alzheimer's disease

We included CSF of 23 (12 men and 11 women) autopsy-confirmed AD patients. In 15 cases of these frontal cortex from autopsy was available. All 23 patients of this group fulfilled the NINCDS–ADRDA (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association) criteria for clinical diagnosis of probable AD (McKhann et al. 1984). Age at the time of LP of this group was 69 ± 15 years. Mean number of days in between lumbar puncture and death of those patients where frontal cortex was available were 263 ± 439 days. Mean disease duration was 25.0 ± 22.8 months.

Control groups

The comparison group was divided into a group of ten patients with clinical diagnoses, whose CSF served as comparison group to the diagnostic groups (CGc) and ten brain samples of patients who died from non-neurological diseases (CGp).

The CGp group consisted of ten patients (5 men and 5 women). Mean age in this group was 76 ± 8 years (mean $\pm$ SD). Eight patients died from heart diseases, one from lung emboli and one from ruptured aneurysm of the

Table 1 Patient demographics

	Diagnostic groups		Control groups	
	Creutzfeldt–Jakob disease (CJD) <i>N</i> = 9	Alzheimer's disease (AD) <i>N</i> = 23	Comparison group (CGc) <i>N</i> = 10	Comparison group (CGp) <i>N</i> = 10
Sex (male:total)	0.44	0.52	0.5	0.5
Age at LP (years)				
Mean $\pm$ SD	61 ± 16	69 ± 15	62 ± 11	76 ± 8
Median (range)	65 (40–80)	72 (58–92)	62 (46–84)	77 (61–88)
Time between LP and death (days)				
Mean $\pm$ SD	222 ± 265	263 ± 439	n.d.	n.d.
Median (range)	58 (30–700)	45 (199–1556)		
Disease duration (months)				
Mean $\pm$ SD	10.5 ± 9.3	25.0 ± 22.8	n.d.	n.d.
Median (range)	9 (2–27)	20 (10–80)		

LP lumbar puncture, SD standard deviation, n.d. not determined

aorta. No clinical signs for neurodegenerative disease and/or dementia were present at lifetime in any of these patients.

The CGc group consisted of ten non-demented patients (5 men and 5 women), who underwent lumbar puncture for other differential diagnostic reasons. None of these patients displayed clinical features of either neurodegenerative disease or dementia. Age of this group was 62 ± 11 years. Mini-Mental Status Examination in all cases showed no cognitive decline (>28 points) (Folstein et al. 1975). The group included patients suffering from peripheral neurological disorders ($n = 7$), depression ($n = 2$), and migraine ($n = 1$). None displayed clinical features of either neurodegenerative disease (including prion disease) or dementia.

Immunohistochemistry

Immunohistochemistry and neuropathological diagnoses were performed as published previously (Bibl et al. 2006; Kretschmar et al. 1996; Schulz-Schaeffer et al. 2000; Wiltfang et al. 2001). Immunohistochemistry was performed with monoclonal antibodies against β -amyloid (β -Amyloid, Clone 6F/3D, 1:50, Dako, Glostrup, Denmark), tau protein (AT8, 1:200, Innogenetics, Gent, Belgium) and prion protein (12F10) (Krasemann et al. 1996). Neuropathological diagnosis was performed using international criteria (Braak and Braak 1991; Kretschmar et al. 1996; Mirra et al. 1991). All neuropathological diagnoses were performed by the German Reference Centres for Spongiform Encephalopathies and Neurodegenerative Diseases in Munich and Goettingen, Germany.

Sample preparation

After informed consent from patients and/or next of kin, CSF was drawn from patients by lumbar puncture in polypropylene vials and centrifuged (1,000g, 10 min, 4°C). Following completion of routine diagnostics, samples were aliquoted. Aliquots of 200 μ l were stored at -80°C within 24 h for subsequent A β -SDS-PAGE/immunoblot analysis. CSF samples were applied to the A β -SDS-PAGE/immunoblot directly.

All patients or their relatives were asked for their agreement to autopsy after death. The brain tissue was used for the study only when informed consent was given.

The RIPA (radio immune precipitation assay)-soluble fraction of A β peptides was extracted from homogenates of the frontal lobe cortex of each patient as follows: ~ 100 mg of human brain was thawed into 1 ml RIPA buffer (50 mM HEPES, pH 7.4, 1% NP40, 0.5% Na-deoxycholate, 0.1% SDS, 150 mM NaCl) which contained protease inhibitors (complete protease inhibitor cocktail, 1 tablet in 10 ml solution). Samples were homogenized in

1.5-ml polypropylene tubes by ultrasonification (2×25 strikes, Bronson sonifier 250).

After centrifugation ($14,000 \times g$ for 10 min at 4°C) the protein content of supernatants was adjusted to 3 mg/ml by dilution with $1 \times$ RIPA buffer. Protein concentration was determined by bicinchoninic acid (BCA) assay. The pellet (RIPA-insoluble fraction) was further extracted using 70% (v/v) formic acid in water, spun as above, and the supernatant was evaporated using a Speed-Vac concentrator, and dissolved in sample buffer.

To detect RIPA-soluble A β peptides, supernatant were immunoprecipitated with 50 μ l of magnetic microparticles (2 μ g mAb 1E8/ 3.36×10^7 DynaBeads) or were directly applied to the gel as published elsewhere (Bibl et al. 2004, 2006; Wiltfang et al. 2001, 2002).

Standard preparations of synthetic A β peptide mixture were created as described previously (Bibl et al. 2004) and bands were quantified from individual blots of each patient relative to this dilution series using a charge coupled device camera (CCD-camera) and the Quantity-ONE software (BIORAD). The inter- and intra-assay coefficients of variation and sensitivity of detection of the A β -SDS-PAGE/immunoblot have been published elsewhere (Bibl et al. 2004; Wiltfang et al. 2002).

We focus on the values of the analyzed peptides relative to the total A β peptide (in %), which has been shown to be more stable and persistent. We show absolute values in Table 2 only for reasons of comparison between CSF and brain.

Statistical analysis was carried out as described previously (Bibl et al. 2004, 2006).

Results

A highly conserved pattern of C terminal-truncated and partially also amino terminal-truncated A β peptides could be shown by the A β -SDS-PAGE/immunoblot in the CSF and brain of all investigated patients.

Age at the time of lumbar puncture (LP), gender and days between LP and autopsy did not differ significantly between the groups ($p > 0.05$).

A β -SDS-PAGE/immunoblot of CSF

The comparison group (CGc) could be significantly distinguished from the AD patients due to the former's relative abundance of A β 1-42% ($p = 1 \times 10^{-6}$) and A β 1-39% ($p = 0.08$).

CSF samples from CJD patients could be differentiated from those of the CGc by the relative amounts of A β 1-42% ($p = 2 \times 10^{-5}$). The other measured A β peptides did not differ significantly from the control groups ($p > 0.05$).

Table 2 Quantitative analysis of the mean A β peptide species 1-42, 1-40 and 1-38 as well as A β total in the intra vitam CSF by A β SDS-PAGE/immunoblot (detection antibody, mAb 1E8) and the correspondent detergent-soluble fraction of immunoprecipitation from brain homogenates (mAb 1E8) of patients with Alzheimer's disease (AD), Creutzfeldt-Jakob disease (CJD) and from controls (CGc and CGp) (mean $\pm$ standard deviation)

Disease	AD	CJD										Comparison group (CG)				
												CGc		CGp		
		A (n = 1)					B (n = 2)					(n = 10)		0 (n = 6)		
CSF	Brain	CSF	Brain	CSF	Brain	CSF	Brain	CSF	Brain	CSF	Brain	CSF	Brain	CSF	Brain	
ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
CERAD	A (n = 1)	B (n = 4)	C (n = 10)	0 (n = 5)	A (n = 2)	B (n = 2)										
A β 1-42	0.27	12.730	0.31 $\pm$ 0.27	16.234 $\pm$ 14.762	0.25 $\pm$ 0.18	24.827 $\pm$ 8.678	0.38 $\pm$ 0.26	0.010 $\pm$ 0.007	0.38 $\pm$ 0.19	22.096 $\pm$ 30.397	0.23 $\pm$ 0.23	2.056 $\pm$ 2.102	1.66 $\pm$ 0.4	0.05 $\pm$ 0.013	4.088	2.056 $\pm$ 2.102
A β 1-40	2.73	0.144	4.371 $\pm$ 1.80	20.482 $\pm$ 21.859	3.54 $\pm$ 2.15	13.529 $\pm$ 22.078	3.24 $\pm$ 2.03	0.016 $\pm$ 0.004	3.4 $\pm$ 1.7	8.744 $\pm$ 12.347	2.86 $\pm$ 3.19	0.061 $\pm$ 0.058	9.12 $\pm$ 2.1	0.016 $\pm$ 0.007	0.141	0.61 $\pm$ 0.58
A β 1-38	0.78	0.098	1.24 $\pm$ 0.90	3.929 $\pm$ 5.113	0.62 $\pm$ 0.27	6.725 $\pm$ 11.193	0.65 $\pm$ 0.39	0.005 $\pm$ 0.004	0.71 $\pm$ 0.35	1.142 $\pm$ 1.502	0.57 $\pm$ 0.57	0.052 $\pm$ 0.037	2.04 $\pm$ 0.48	0.006 $\pm$ 0.006	0.352	0.52 $\pm$ 0.37
A β total	4.8	15.377	7.55 $\pm$ 3.70	46.193 $\pm$ 42.224	5.33 $\pm$ 2.97	61.436 $\pm$ 51.914	5.10 $\pm$ 3.25	0.031 $\pm$ 0.009	5.37 $\pm$ 2.61	36.310 $\pm$ 50.339	4.32 $\pm$ 4.61	2.723 $\pm$ 2.885	15.19 $\pm$ 3.36	0.088 $\pm$ 0.168	5.581	2.723 $\pm$ 2.885

In the brains the occurrence of neuritic plaque pathology was semi quantitatively determined according to the classification (age-related plaque scores 0, A, B, C) of The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra et al. 1991)

Comparing AD to CJD, decreased levels of CSF A β 1-42% ($p = 0.006$) were observed in AD but this was not the case for any other measured CSF A β peptide ($p > 0.05$). The previously described ratios of A β 1-42/A β 1-39 (Wiltfang et al. 2003) and A β 1-42/A β 1-37 (Bibl et al. 2006) were highly significantly elevated in CGc compared to the analyzed CJD group ($p = 2 \times 10^{-4}$ and $p = 6 \times 10^{-4}$) and AD group ($p = 1 \times 10^{-6}$ and $p = 2 \times 10^{-5}$) (Fig. 1).

The absolute sum of all investigated A β peptides (A β total) was significantly higher in AD but not in CJD compared to the controls CGc ($p < 0.001$ and $p > 0.05$, respectively).

In detail, the comparison group could be very significantly differentiated from patients suffering from AD based on the AD group's decreased absolute levels of A β 1-42 ($p = 0.007$), A β 1-40 ($p = 1 \times 10^{-5}$), and A β 1-38 ($p = 0.0007$). However, the other analyzed peptides did not differ significantly between AD and CGc ($p > 0.05$).

A β -SDS-PAGE/immunoblot of brain

Brain homogenates from the analyzed CJD patients displayed no significant differences in any investigated A β peptide in comparison with CGp ($p > 0.05$).

AD patients compared to CJD patients and controls showed significantly higher absolute levels of A β 1-42 ($p = 0.002$), A β 1-40 ($p = 8 \times 10^{-4}$), and A β 1-38 ($p = 0.002$).

The characteristics of the diagnostic groups relative to the control group with regard to the amounts of each single A β peptide relative to the total amount of measured A β -peptides are summarized in Table 2.

Amino-terminal-truncated A β 2-42 and A β 2-40 were analyzed in all brain homogenates; A β 2-42 could be detected in all AD and in five CJD cases as well as in four cases of the comparison group (CGp). Mean levels of A β 2-42 ranged AD > CJD > CGp. A β 2-40 was only detectable in 4 AD brain homogenates (2 CERAD B, 2 CERAD C) but not in any other group.

The amount of A β peptides in brain homogenates increased with increasing plaque pathology quantified by CERAD classification in all groups. This correlation of A β peptides was significant for A β 1-42% and A β 1-40% ($p < 0.05$). Interestingly, in CSF A β 1-42% and A β 1-38% correlated significantly with the CERAD classification for CJD and AD ($p < 0.05$; Table 2).

The differences between the RIPA- and formic acid-extracted brain homogenates remained subtle.

Discussion

We analyzed the A β peptide pattern in CSF and concomitant brain homogenates of patients with neuropathologically

Fig. 1 Logarithmic box-plots of relative levels of A β -peptides in CSF of the diagnostic (CJD and AD) and the comparison groups (CGc)

confirmed CJD, AD and in controls by A β -SDS-PAGE immunoblot to identify disease-specific changes and possible relations between the relative abundances of A β peptides in straight CSF and the RIPA- as well as formic acid-soluble fraction of brain homogenates. We correlated our findings with the occurrence of neuritic amyloid plaques in the brain.

We consistently observed a highly conserved pattern of A β peptides in all analyzed CSF and brain specimens.

In brain homogenates we could show a positive correlation between A β -plaques indicated by CERAD classification and the amount of analyzed A β peptides 1-42, 1-40, 1-38, 2-42 and total A β peptides.

We confirmed for the first time the different CSF A β peptide pattern in neuropathologically verified AD and CJD patients using our A β -SDS-PAGE immunoblot approach. We could reconfirm our previous findings with a selective reduction of A β 1-42% as well as a decrease in the ratios A β 1-42/1-39 and A β 1-42/1-37 in autopsy-confirmed CJD and AD patients (Bibl et al. 2006; Wiltfang et al. 2003).

Several mechanisms may be responsible for the prominent decrease of CSF A β 1-42 in AD: First of all, the reduction of CSF A β 1-42 levels in AD does reflect A β -plaque pathology. This issue has recently been emphasized in vivo by the inverse relation of CSF A β 1-42 and amyloid imaging (Fagan et al. 2006). However, it is unlikely that this mechanism accounts for the decrease in CJD patients especially as here no prominent A β -plaque pathology is

observed (Otto et al. 2000; Wiltfang et al. 2003). With our study, we are able to confirm this decrease of CSF A β 1-42 in neuropathologically verified CJD patients and show directly that this decrease occurs irrespective of the plaque load. We are now also able to show that no other A β -peptide fraction, even those not accessible to ELISA or immunohistochemistry, can be responsible for this decrease.

One idea put forward is that the presence of a putative carrier causes a high binding of affinity and masking of epitopes in A β 1-42 (Otto et al. 2000; Wiltfang et al. 2003).

Other studies indicate the existence of alternative mechanisms, including the formation of SDS-stable oligomers (Jarrett et al. 1993) and supramolecular aggregates of A β peptides (Pitschke et al. 1998). However, such fractions cannot be seen by our method. Alternatively, it might be hypothesized that in CJD, A β -peptides in CSF are additionally cleaved, leading to a decrease of A β 1-42 in CSF.

CJD is mainly characterized by a diffuse synaptic, a perivacuolar, a perineuronal or a plaque-like deposition pattern of misfolded prion proteins (PrP^{Sc}) in the brain. In rare cases, prion plaques can be found. It was even shown that the physiological prion protein (PrP^C) promotes A β -plaque formation by enhancing A β aggregation (Parkin et al. 2007; Schwarze-Eicker et al. 2005). Thus PrP^C pathology may influence the A β metabolism and presence of A β peptides in CSF, which needs to be investigated further.

We did not find any correlation between the analyzed A β peptides and the polymorphism at codon 129 of the prion protein gene in the subgroup of 9 CJD and 11 AD patients, both of which were too small for further statistical evaluation (data not shown).

The fact that we investigated brain homogenates derived from the gyrus frontalis medialis and no other brain regions might be considered a weakness of our study, however, we chose this area as it was shown to be particularly sensitive to aging and plaque pathology (Tapp et al. 2004).

We can emphasize that the decrease of CSF A β 1-42 is indeed negatively correlated with the A β plaque load, at least in AD brains. However, for CJD this relation is less clear. The decrease in CSF A β 1-42 levels in CJD remains unresolved and should be further investigated.

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