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S-adenosylmethionine is decreased in the cerebrospinal fluid of patients with Alzheimer’s disease

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Running title: CSF levels of SAM in AD

Keywords: Alzheimer dementia; homocysteine metabolism; cerebrospinal fluid
Abstract

Increased plasma homocysteine levels have been described as an independent risk factor for Alzheimer dementia (AD), but the underlying pathophysiology is unclear. This single center cross sectional, correlational study analyzed the homocysteine metabolism in 60 AD patients and 60 control subjects. Fasting plasma levels of vitamin B12, folate and homocysteine as well as cerebrospinal fluid (CSF) levels of folate derivates, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and homocysteine were measured. In addition, the apolipoprotein E (APOE) genotype was determined. As expected, the APOE4 allele was significantly over-represented in AD patients compared with controls (p<0.001).

Homocysteine plasma levels of the highest quartile were more frequent in the patients than in the controls (p=0.008). In addition, AD patients had significantly lower CSF levels of the methyl group donor SAM (193±31nmol/L vs. 207±37nmol/L; p=0.032). Accordingly, the SAM/SAH ratio, which represents the methylation capacity, was significantly lower in the CSF of the AD patients (7.6±2.4 vs. 9.1±2.8; p=0.003). Further, explorative analysis of all subjects showed that CSF SAM levels were lower in carriers of the APOE4 allele compared with non-carriers (189±30nmol/L vs. 207±36nmol/L; p=0.010). Of the individuals with CSF SAM levels of the lowest quartile, 63% carried the APOE4 allele compared with 17% of the individuals with CSF SAM levels of the highest quartile (Pearson: $\chi^2=9.9$; p=0.002; OR: 0.126 with 95%CI = 0.32-0.49). These data suggest that AD is associated with lower CSF SAM levels and that this is at least partly due to an association of the APOE4 allele with reduced SAM levels in the CSF.
Introduction

Sporadic Alzheimer’s disease (AD) is the most common cause of dementia in the elderly. Known risk factors for AD include age, gender, the presence of the apolipoprotein epsilon 4 (APOE4) allele and increased homocysteine plasma levels [1, 2]. Hyperhomocysteinemia, often related to folate or vitamin B12 deficiency, is a common finding in the elderly [3] and is associated with cognitive impairment and cognitive decline [4, 5]. The association between hyperhomocysteinemia and AD is well established, however, the underlying pathophysiology remains unexplained. In particular, it is not known whether homocysteine level itself or other components of the homocysteine metabolism play a role in the development of AD. Recent studies in cell culture experiments and mouse models suggested that two metabolites of the homocysteine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) may be important in Alzheimer pathogenesis, e. g. by influencing expression of presenilin 1 and β-secretase, leading to an increase in Aβ production [6-8]. S-adenosylmethionine (SAM), serves as ubiquitous methyl- group donor and is necessary e.g. for the synthesis of neurotransmitters, neuronal membrane stability and DNA- methylation [9, 10]. After transmethylation, the resulting degradation product of SAM is S-adenosylhomocysteine (SAH), which is hydrolyzed to homocysteine (see figure 1).

A few studies have investigated the association of parameters of homocysteine metabolism with AD in the cerebrospinal fluid of patients (CSF). The comparison of CSF of eight AD patients and six control subjects revealed higher CSF homocysteine in the AD group [11]. In contrast, another study investigating CSF of 83 control subjects and 38 AD patients found that homocysteine levels increased with age, but were not associated with AD [12]. Concerning SAM and SAH, Mulder and co-workers investigated the CSF of 30 AD patients and 28 controls [13] and did not observe any significant differences of these parameters between the two groups studied. However, this study may have been limited by the fact that 20 of the controls also presented “subjective memory complaints“, yet without diagnosis of AD.
Another study reported lower SAM CSF levels in nine patients with AD compared with 29 neurological disease control subjects [14].

In the present study we compared the fasting CSF levels of SAM, SAH, homocysteine and folate fractions as well as fasting plasma levels of folate, vitamin B12 and homocysteine of 60 AD patients with 60 controls without cognitive impairment to further investigate the association of the homocysteine metabolism with AD.

**Materials and Methods**

The 60 Caucasian study participants with AD were referred to the Memory Clinic, Department of Psychiatry, University of Bonn, for investigation of their cognitive complaints. They met clinical diagnostic criteria for probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and Related Disorders Association [15] and DSM-IV criteria for dementia of the Alzheimer type.

AD diagnosis was based on neuropsychological and clinical evaluation including mini-mental state examination (MMSE) and was approved by a consensus conference of psychiatrists and neuropsychologists prior to the metabolites measurements. The presence of relevant vascular cerebral damage was excluded for all study participants with AD by computed tomography or magnet resonance tomography and the Hachinsky Ischemic Score (score<4) [16]. Blood and CSF samples of all patients were obtained at the diagnostic work-up in which diagnosis of AD was first made, thus, the time that the samples were collected was the same as the age at diagnosis.

As disease controls, we recruited 60 consecutive Caucasian patients who underwent lumbar puncture at the Department of Neurology, University of Bonn, for different indications such as exclusion of CNS inflammation, exclusion of aneurismal subarachnoid hemorrhage or
exclusion of meningitis. All controls were assessed with a Mini-Mental State Examination (MMSE). Exclusion criteria included age under 18 years, history or evidence of cognitive decline as assessed with the MMSE [17], subjective mental disorders, regular intake of vitamin supplements, neurodegenerative or inflammatory diseases of the central nervous system, and other severe or unstable illness such as symptomatic cardiac disease, renal or hepatic dysfunction, insulin-dependent diabetes mellitus, untreated thyroidal dysfunction, or excessive alcohol intake. In addition, CSF samples indicating blood CSF barrier disturbances or inflammatory signs, defined as CSF whole protein content >500mg/dl and more than 5 leucocytes/mm³, were excluded from both the AD group and controls. Diagnostic lumbar punctures were performed at the Departments of Neurology or Psychiatry, University of Bonn. Lumbar puncture belonged to the diagnostic work-up within the clinical routine in all cases of dementia and was not primarily done for study purposes. A standardized technique with a 20G “atraumatic” spinal needle and a sitting or lying position for the patient was applied. CSF samples were immediately put on dry ice and then stored at -80 °C until assay procedures. Fasting plasma homocysteine concentrations were measured by means of particle-enhanced immunonephelometry with a BN II System (Siemens Healthcare Diagnostics, Eschborn, Germany). Fasting serum vitamin B12 and folate concentrations were measured by means of a competitive chemiluminescent immunoassay with an Access™ Immunoassay System (Beckman Coulter, Krefeld, Germany). Deproteinized serum samples for the analysis of SAM and SAH were not available. CFS was analyzed by tandem mass spectrometry for folate derivates (5-methyltetrahydrofolate = 5-MTHF, 5,10-methylenetetrahydrofolate = 5,10-MTHF, 5-formyl-tetrahydrofolate = 5-formyl-THF, tetrahydrofolate = THF, folic acid), SAM and SAH and homocysteine as described previously [18, 19]. Homocysteine was measured in CSF by HPLC using fluorescence detection as previously described [20]. Leukocyte genomic DNA was isolated with the Qiagen blood isolation kit (Qiagen, Hilden, Germany). The APOE genotype was determined as previously described [21].
For statistical analysis, univariate analysis of variance (ANOVA) was used to compare CSF and blood parameters between AD patients and controls. CSF SAM levels between APOE4 carriers and APOE4 non-carriers were compared by independent samples t-test. As age and gender significantly differed between patients and controls, the results obtained from ANOVA analysis were retested by multivariate nominal regression analysis with age and gender as covariables for adjustment of differences. Due to unknown interactions of the blood and the CSF parameters, the multivariate analysis was done separately for blood and CSF parameters. The association of the APOE4 allele with diagnosis AD was analyzed with Pearson’s χ² test. Alpha was set as two-sided 0.05. Plasma and CSF levels of homocysteine were compared by Pearson’s bivariate analysis of correlation. The study was approved by the local ethics committee. Written informed consent was obtained from all study participants or their trustee.

Results

We compared samples of 60 AD patients (years at diagnosis ± standard deviation: 73±8 years; 43 women) with samples of 60 controls (62±10 years; 24 women). Mean MMSE of controls was 29.0 (95% confidence interval: 28.3-29.7) in comparison to 21.5 (20.5-22.5) of the AD patients (ANOVA: F=152; p<0.001). As expected, the APOE4 allele was significantly over-represented in AD patients compared with controls (87% vs. 27% carriers; Pearson’s Chi² test: χ²=38.0; p<0.001). We did not observe significant differences between AD patients and controls concerning mean plasma levels of homocysteine, vitamin B12, total folate or creatinine (table 1).

In the CSF, 5-MTHF (5-methyltetrahydrofolate) was present in higher concentrations than the quantification limit in all samples, 5,10-MTHF (5,10-methylenetetrahydrofolate) was detectable in 18 samples, formyl-THF (formyltetrahydrofolate) and THF (tetrahydrofolate) were detectable in fewer than 10 samples. Folic acid, the synthetic form used for folate
supplementations was not detectable in any of the samples. The quantification limit for the cumulative group of the folate fractions was approximately 0.4nmol/l. There was no difference in CSF levels of 5-MTHF ($F=0.54; p=0.464$), 5,10-MTHF ($F=1.54; p=0.218$), formyl-THF ($F=0.31; p=0.580$) and THF ($F=0.05; p=0.818$) between AD patients and controls. Because of the limited number of data, the OR of quartiles was not calculated for CSF folate fractions.

Whereas there was no significant difference in CSF or plasma homocysteine levels between patients and controls, the frequency of homocysteine plasma levels of the highest quartile was significantly higher in the patients than in the controls ($p=0.008$; table 1). Accordingly, homocysteine plasma levels of the highest quartile were associated with AD in multivariate nominal regression analysis with age and gender as covariables and with diagnosis AD versus control as dependent variable ($p=0.023$). AD patients showed significantly lower CSF levels of SAM ($p=0.032$) and significantly higher CSF levels of SAH ($p=0.037$) by ANOVA. Accordingly, the SAM/SAH ratio was significantly lower in the AD patients ($p=0.003$). When we retested these results multivariate regression analysis with age and gender as covariables, the difference of SAM between patients and controls was confirmed ($Wald=3.81; p=0.048$), whereas the difference of SAH was not ($Wald=0.022; p=0.961$).

As the APOE genotype is a major risk factor for AD, we next tested whether the observed associations of metabolites with AD were independent from the APOE genotype. When the APOE genotype was added as covariable to the multivariate model, regression analysis showed no association of AD with SAM levels ($Wald=0.460; p=0.543$). Thus, we suspected that CSF levels of SAM were associated with the APOE genotype, and we additionally tested metabolite levels in strata of the APOE genotype. While the other parameters showed no significant differences, CSF SAM levels were significantly lower in the carriers of the APOE4 allele (ANOVA: $F=6.93; p=0.010$ for patients plus controls). This association was
significant when patients and controls were analyzed together (table 2), but not when patients and controls were analyzed separately. Of the individuals with CSF SAM levels of the lowest quartile, 63% carried the APOE4 allele compared with 17% of the individuals with CSF SAM levels of the highest quartile (Pearson: $\chi^2 = 9.9; p = 0.002$; OR: 0.126 with 95% CI = 0.32-0.49).

**Table 1:** Blood and CSF parameters in AD patients and controls

<table>
<thead>
<tr>
<th>parameter</th>
<th>AD patients (n=60)</th>
<th>controls (n=60)</th>
<th>ANOVA</th>
<th>OR quartiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>homocysteine</td>
<td>14.1±4.3 (12.9-15.3)</td>
<td>12.7±5.4 (11.3-14.1)</td>
<td>F=2.20; p=0.144</td>
<td>4.889 (1.513-15.793); p=0.008</td>
</tr>
<tr>
<td>(plasma; µmol/L)</td>
<td></td>
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<tr>
<td>vitamin B12</td>
<td>272±192 (219-323)</td>
<td>248±103 (221-276)</td>
<td>F=0.636; p=0.427</td>
<td>1.00 (0.350-2.859); p=0.989</td>
</tr>
<tr>
<td>(serum; pmol/l)</td>
<td></td>
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<tr>
<td>total folates</td>
<td>15.62±7.04 (13.69-17.52)</td>
<td>14.05±7.74 (11.99-16.09)</td>
<td>F=1.24; p=0.269</td>
<td>2.500 (0.828-7.548); p=0.104</td>
</tr>
<tr>
<td>(serum; nmol/l)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>creatinine</td>
<td>74.3±13.3 (69.9-77.9)</td>
<td>89.4±77.4 (66.4-111.5)</td>
<td>F=1.73; p=0.191</td>
<td>0.650 (0.226-1.866); p=0.423</td>
</tr>
<tr>
<td>(plasma; µmol/L)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SAM (CSF; nmol/L)</td>
<td>193±31 (184-201)</td>
<td>207±37 (197-217)</td>
<td>$F=4.74; p=0.032$</td>
<td>0.222 (0.072-0.686); p=0.009</td>
</tr>
<tr>
<td>SAH (CSF; nmol/L)</td>
<td>26.9±6.2 (25.2-28.5)</td>
<td>24.3±6.8 (22.5-26.1)</td>
<td>$F=4.44, p=0.037$</td>
<td>3.333 (1.098-10.116); p=0.034</td>
</tr>
<tr>
<td>SAM/SAH (CSF)</td>
<td>7.6±2.4 (6.9-8.3)</td>
<td>9.1±2.8 (8.4-9.9)</td>
<td>$F=9.92; p=0.003$</td>
<td>0.152 (0.048-0.484); p=0.001</td>
</tr>
<tr>
<td>homocysteine (CSF; nmol/l)</td>
<td>71.9±43.5 (59.9-83.9)</td>
<td>77.6±52.6 (63.8-91.4)</td>
<td>F=0.39; p=0.536</td>
<td>0.750 (0.262-2.150); p=0.592</td>
</tr>
</tbody>
</table>

Mean ± 1 standard deviation and 95% confidence intervals (in brackets) are given. OR quartiles: Mantel-Haenszel Common Odds Ratio estimates with asymptomatic 95% confidence intervals (in brackets) and asymptomatic two-sided significance are given for the distribution of the highest and the lowest quartile of the respective parameter between AD patients and controls. An OR lower than 1.0 means that the lowest quartile was over-represented in the AD group.
Table 2: CSF levels of SAM in dependency on the APOE4 genotype

<table>
<thead>
<tr>
<th></th>
<th>APOE4 present</th>
<th>APOE4 absent</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>all</td>
<td>189±30 (n=50)</td>
<td>207±36 (n=70)</td>
<td>t=2.63; p=0.010</td>
</tr>
<tr>
<td>60 AD patients</td>
<td>188±29 (n=43)</td>
<td>199±36 (n=17)</td>
<td>t=1.10; p=0.240</td>
</tr>
<tr>
<td>60 controls</td>
<td>198±34 (n=7)</td>
<td>210±36 (n=53)</td>
<td>t=0.72; p=0.474</td>
</tr>
</tbody>
</table>

In a generalized linear model, there was no significant interaction between AD-diagnosis and the APOE4 allele on SAM levels (not shown). SAM did not correlate with mini mental state examination in the AD patients.

Discussion

In the present study, several parameters of homocysteine metabolism were analyzed in the CSF and plasma of 60 AD patients and 60 control subjects without cognitive impairment. Due to differences in age and gender between patients and controls as limitation, we performed multivariate analysis with age and gender as covariables in addition to univariate analyses. Whereas homocysteine plasma levels were not significantly associated with AD in ANOVA, individuals with homocysteine plasma levels of the highest quartile significantly more often belonged to the AD group in comparison to individuals with homocysteine levels of the lowest quartile. This is in line with the known association of elevated homocysteine plasma levels and AD [1]. However, we did not observe higher homocysteine CSF levels in AD patients like previously reported [22].

In addition, we did not detect significant differences in blood levels or quartiles of vitamin B12, folate or creatinine, nor in CSF levels or quartiles of folate fractions. However, in the AD patients, we observed significantly lower CSF levels of SAM and a significant reduction of the SAM/SAH ratio. As the observed reduction of SAM in CSF was also significant in a
multivariate model, this difference is unlikely to be explained by differences of age and
gender in patients and controls. This confirms the previous report by Bottiglieri et al. [14],
who observed lower CSF levels of SAM in nine AD patients in comparison to 29 patients
with other neurological diseases. Accordingly, in a post-mortem analysis, the concentration of
SAM was severely decreased in the brains of AD patients [23].
Due to the role of SAM as ubiquitous methyl group donor and SAH as a strong inhibitor of
SAM-dependent transmethylation reactions, lower levels of SAM and higher levels of SAH
are supposed to result in a reduced methylation capacity [24]. It has previously been
suggested that low SAM levels are associated with DNA-demethylation followed by
increased expression of presenilin 1 and β-secretase, leading to an increase in Aβ production.
Supplementation of SAM prevented these changes in cell culture experiments and mouse
models [6-8]. Additionally, high concentrations of the hyperphosphorylated tau protein (P-
tau) in CSF, which predict the development of dementia [25], were associated with a low
activity of the SAM-dependent protein phosphatase PP2A [26]. Thus, low brain or CSF levels
of SAM and high levels of the SAM-inhibitor SAH might promote both Aβ production and P-
tau accumulation. Another aspect of a possible association of methionine metabolism with
neurodegenerative disorders is oxidative stress: The transsulfuration reaction of homocysteine
to cysteine, a precursor of glutathione, is activated by SAM [9]. Therefore, a lack of SAM
may result in a reduced antioxidative capacity and increased oxidative stress, a hallmark of
several neurodegenerative conditions including AD [27, 28]. Recent findings showed that oral
substitution of SAM results in an increase in both plasma and CSF levels of SAM, and SAM
substitution may provide some clinical improvement in AD patients [29].
The second interesting observation in our study was the association of CSF levels of SAM
with the APOE4 allele. Knowing that these results are limited by the small sizes of the
respective subgroups and by the limitations of pooling patients and controls for such analyses,
the association of SAM levels with the APOE4 allele was not significant in separate analyses
of patients and controls, although both (as yet) healthy and demented carriers of the APOE4 allele had lower levels of SAM than non-carriers. This observation leads to the question whether there is a biological effect of APOE on SAM, which remains speculative.
Acknowledgments

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References


The sulfur-containing amino acid methionine is activated to S-adenosylmethionine (SAM), which is an ubiquitous methyl group donor. Degradation product of SAM is S-adenosylhomocysteine (SAH), which is hydrolyzed to homocysteine. Homocysteine can be remethylated to methionine and SAM, which depends on derivates of folate (5, 10-MTHF, 5-MTHF) and vitamin B12 as cofactors. Alternatively, homocysteine can be transsulfurated to cysteine as component of glutathione, which depends on vitamin B6 as cofactor.