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Alzheimer's disease is not associated with altered concentrations of the nitric oxide synthase inhibitor asymmetric dimethylarginine in cerebrospinal fluid

Short Communication

C. Mulder[1], **L.-O. Wahlund**[3], **M. Blomberg**[3], **S. de Jong**[1], **G. J. van Kamp**[1], **P. Scheltens**[2], and **T. Teerlink**[1]

Departments of [1] Clinical Chemistry, and
[2] Neurology, VU University Medical Center, Amsterdam, The Netherlands
[3] Department of Clinical Neuroscience, Section of Geriatric Medicine, Huddinge University Hospital, Karolinska Institute, Huddinge, Sweden

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Summary. Nitric oxide (NO) may play a role in the pathophysiology of Alzheimer's disease (AD). Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, is involved in regulation of NO production. Recently it has been reported that dimethylarginine dimethylaminohydrolase, an enzyme that hydrolyses ADMA into citrulline and dimethylamine, is specifically elevated in neurons displaying cytoskeletal abnormalities and oxidative stress in AD. We hypothesized that this could lead to altered CSF concentrations of ADMA in AD. Measurement of ADMA and dimethylamine in CSF revealed no significant differences between AD patients (n = 20) and age-matched control subjects (n = 20). Our results suggest that in early stages of AD overall regulation of NO production by ADMA is not aberrant.

Keywords: ADMA, Alzheimer's disease, DDAH, dimethylamine, nitric oxide.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of unknown etiology. Neuropathological hallmarks of AD are senile plaques and neurofibrillary tangles. The relationship between the observed lesions in the brain and the AD disease process has long been debated. Two broad hypotheses about the mechanism have emerged (Selkoe, 1999; Lannfelt, 1997). Ac-

cording to the *amyloid cascade hypothesis*, both familial and sporadic variants of AD are caused by amyloid accumulation, especially $A\beta_{1-42}$, in the brain. Overproduction of $A\beta_{1-42}$ or failure to clear this peptide leads to AD, primarily through amyloid deposition associated with cell death. According to the *inflammatory and neurotoxic cascade hypothesis*, the damaged neurons, highly insoluble $A\beta_{1-42}$ deposits, and neurofibrillary tangles provide stimuli for inflammation, leading to local upregulation of complement, cytokines, acute phase reactants, and other inflammatory mediators (Eikelenboom and Veerhuis, 1999). Nitric oxide (NO), derived from arginine by nitric oxide synthase (NOS), plays an important role as vasodilator and neurotransmitter, and is involved in cellular immune response. In addition, NO can react with superoxide to give the very reactive peroxynitrite that causes oxidative damage to proteins by nitration of tyrosine residues and may lead to cellular injury (Wiesinger, 2001). There is ample evidence for the presence of nitrotyrosine modified proteins in AD (Su et al., 1997; Smith et al., 1997; Hensley et al., 1998).

Methylation of proteins at arginine residues by protein-arginine methyltransferases is involved in the modulation of protein-nucleic acid interactions (Gary and Clarke, 1998). The main methylation product is asymmetric dimethylarginine (ADMA), in which both methyl groups are attached to the same terminal guanidino nitrogen. Symmetric dimethylarginine (SDMA), in which both terminal nitrogen atoms of the guanidino group are methylated, is formed in lower amounts, but is especially abundant in myelin basic protein (Gary and Clarke, 1998). Both ADMA and SDMA are released into the cytoplasm following proteolysis. Intracellular ADMA inhibits NOS activity, whereas SDMA is not inhibitory (Leiper and Vallance, 1999; Vallance et al., 1992). Recently it has been shown that even slightly elevated plasma concentrations of ADMA are associated with an increased risk of acute coronary events, underlining the clinical relevance of ADMA (Valkonen et al., 2001).

Many tissues contain the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyses ADMA into citrulline and dimethylamine (Ogawa et al., 1987; Leiper et al., 1999; Vallance, 2001). DDAH may therefore augment NO synthesis, by relief of NOS inhibition. DDAH has been isolated from bovine brain (Bogumil et al., 1998), and one isoform of human DDAH predominates in tissues that express neuronal NOS (Leiper et al., 1999). Upregulation of mRNA of DDAH after nerve injury has been described (Nakagomi et al., 1999). It was recently demonstrated that in AD patients DDAH is specifically elevated in neurons displaying cytoskeletal abnormalities and oxidative stress pathology, whereas it was undetectable in the neurons of age-matched healthy controls (Smith et al., 1998). Increased DDAH activity in AD, by lowering ADMA concentrations, could lead to increased NO production (Fig. 1) and consequently to NO-mediated oxidative damage. We have tested whether changes in DDAH activity are reflected by altered concentrations of ADMA and its breakdown product dimethylamine in CSF of AD patients compared to age-matched control subjects.

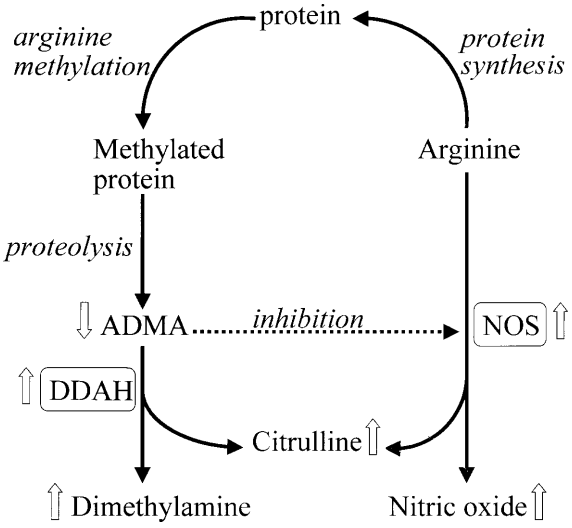

Fig. 1. Schematic representation of the interrelation between the ADMA/DDAH pathway and the arginine/nitric oxide system. ADMA is synthesized by methylation of arginine residues in proteins and is released during protein turnover. Upregulation of DDAH leads to increased degradation of ADMA into citrulline and dimethylamine. As a consequence of decreased ADMA concentrations, inhibition of NOS is relieved, resulting in increased synthesis of nitric oxide. Open arrows indicate changes in enzyme activities and concentrations of metabolites when DDAH activity is upregulated

Materials and methods

Patients and controls

Twenty patients were recruited from the Memory Clinic at Huddinge University Hospital, Sweden. All fulfilled the DSM-IV criteria for dementia and NTNCDS-ADRDA criteria for 'probable' AD. Twenty controls were recruited from the Swedish Pensioner Society and spouses of the patients. Cognitive function was assessed by mini mental state examination (MMSE) score. The lower limit of MMSE for the controls was set at 26 points. Patients and controls went through an extensive dementia investigation including physical examination, MRI, SPECT, EEC, neuropsychological tests as well as blood and urine laboratory tests. The final clinical diagnosis was confirmed after 6 months follow-up. Characteristics of AD cases and controls are shown in Table 1. The study was approved by the institutional ethics committee.

Table 1. Characteristics of AD cases and controls

	AD cases	Controls	P
Number of subjects	20	20	
Gender (M/F)	9/11	7/13	NS
Age, years (mean ± SD)	69.3 ± 8.4	68.8 ± 7.8	NS
MMSE score (median [range])	20.5 [7–26]	29 [27–30]	<0.001
Arginine, μmol/L (mean ± SD)	26.0 ± 3.9	25.7 ± 4.0	0.79
ADMA, μmol/L (mean ± SD)	0.066 ± 0.012	0.065 ± 0.010	0.91
SDMA, μmol/L (mean ± SD)	0.161 ± 0.030	0.153 ± 0.031	0.40
Dimethylamine, μmol/L (mean ± SD)	2.37 ± 0.67	2.01 ± 0.63	0.095

CSF analysis

CSF was obtained by lumbar puncture in the L3/L4 or L4/L5 interspace. The samples were collected in the forenoon with the patient sitting in an upright position. The samples were centrifuged at 1,000 rpm for 10 min, aliquoted in 1 mL fractions and then immediately frozen at −70°C and stored until assayed.

Concentrations of $A\beta_{1-42}$ and tau were determined by sandwich ELISA kits (Innogenetics, Gent, Belgium).

Arginine, ADMA, and SDMA in CSF were measured simultaneously by HPLC using derivatization with ortho-phtaldialdehyde and fluorescence detection (Teerlink et al., 2002). All samples were analyzed in a single analytical series. The intra-assay precision for arginine, ADMA, and SDMA was 0.4%, 1.2%, and 0.8%, respectively.

Dimethylamine was analyzed by reversed-phase HPLC using derivatization with fluorenylmethyl chloroformate and fluorescence detection (Teerlink et al., 1997). Both intra-assay and inter-assay precision were better than 6%.

Statistics

Comparison of patients and controls was performed by t-test, after log-transformation of parameters with skewed distribution. For MMSE scores the Mann-Whitney test was used, and for the M/F distribution we applied the Chi-square test. The level of statistical significance was set at 0.05.

Results

In CSF of the AD patients, decreased concentrations of $A\beta_{1-42}$ compared to controls were found (364 ± 136 versus 830 ± 350 ng/L; $P = 0.001$). Concentrations of tau were increased in CSF of AD patients (558 ± 320 versus 378 ± 179 ng/L; $P = 0.036$). These biochemical data corroborate earlier findings and confirm classification of AD patients and controls by clinical examination with physical as well as neuropsychological tests. No differences between AD patients and controls with respect to CSF concentrations of arginine, ADMA, SDMA, and dimethylamine were observed (Table 1).

Discussion

The main finding of our study is that CSF concentrations of the NOS inhibitor ADMA and its hydrolysis product dimethylamine in AD patients are not different from concentrations in age-matched control subjects. In addition, concentrations of arginine, the substrate of NOS, and SDMA did not differ between AD patients and controls. Therefore, our results suggest that in AD there are no alterations of NOS activity on the concentration of either substrate or the endogenous inhibitor ADMA.

Our study was based on the observation (Smith et al., 1998) that in AD patients the concentration of DDAH is increased in the cytoplasm of neurons with cytoskeletal pathology, whereas enzyme immunoreactivity was undetectable in the neurons of control subjects. Increased DDAH activity could lead to enhanced degradation of ADMA, resulting in increased NO production. We have tested this hypothesis by measuring ADMA and related compounds in CSF. As there are no diffusional barriers between the two compartments, CSF can be regarded as an extension of the intracellular fluid of the brain, and

its composition resembles brain interstitial fluid (Klein, 2000). We developed a novel HPLC technique for the accurate measurement of arginine, ADMA and SDMA, with high sensitivity, allowing analysis of 0.2 mL volumes of CSF. The concentrations of CSF arginine are in close agreement with data obtained by amino acid analysis previously reported by us (Kuiper et al., 2000). CSF concentrations of ADMA were approximately twofold higher than values reported recently for healthy controls (Abe et al., 2001). For SDMA and dimethylamine no literature data on CSF concentrations in AD are available. In this study we found that in CSF SDMA concentrations are 2–3 fold higher than ADMA concentrations, whereas in human plasma approximately equimolar amounts of ADMA and SDMA are present, i.e. 0.42 ± 0.06 μmol/L ADMA and 0.47 ± 0.08 μmol/L SDMA (Teerlink et al., 2002). This high SDMA/ADMA ratio in CSF is in line with the fact that myelin basic protein is a major source of SDMA but not ADMA (Brahms et al., 2001; Gary and Clarke, 1998).

Obviously, increased expression of DDAH in neurons of AD patients could lead to decreased ADMA concentrations. On the other hand, it is also conceivable that DDAH is upregulated as a response to increased ADMA concentrations. In either case the increased DDAH activity would lead to an increased dimethylamine production, which was not found in our study. Increased ADMA production could result from accelerated proteolysis associated with cell death and oxidative damage in AD. If this were the case, however, SDMA concentrations would probably also be increased, which we did not observe.

Our results do not corroborate recently reported data, showing reduced ADMA concentrations in AD patients compared to controls (Abe et al., 2001). This discrepancy may be caused by the selection of patients, as Abe et al. included moderate to severe AD patients in their study (median MMSE = 11.5), while we investigated mild to moderate AD patients (median MMSE = 20.5).

In conclusion, our results do not suggest a generalized aberrant role for the ADMA/DDAH system in the regulation of NO synthesis in early stages of AD.

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Authors' address: Dr. T. Teerlink, Metabolic Unit, Department of Clinical Chemistry, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands, e-mail: t.teerlink@vumc.nl