Content of Autoantibodies to Bradykinin and β-Amyloid$_{1-42}$ as a Criterion for Biochemical Differences between Alzheimer's Dementias

M. A. Myagkova, S. I. Gavrilova*, N. N. Lermontova, Ya. B. Kalyn*, N. D. Selezneva*, G. A. Zharikov*, I. V. Kolykhalov*, T. V. Abramenko, T. P. Serkova, and S. O. Bachurin

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We measured serum content of autoantibodies to β-amyloid protein Aβ$_{1-42}$, its neurotoxic fragment Aβ$_{25-35}$, vasopressin, bradykinin, thrombin, antithrombin III, α$_2$-macroglobulin, and angiotensin II in patients with various forms of Alzheimer's dementias, including presenile and senile dementias of the Alzheimer type. The ratio of antibradykinin and anti-Aβ$_{1-42}$ autoantibody contents differed by 39% in these patients. Our results can be used for the development of a new biochemical method for differential diagnostics of dementias of the Alzheimer type.

Key Words: *Alzheimer's dementias; autoantibodies; Aβ$_{1-42}$; Aβ$_{25-35}$; peptides*

According to modern views, dementias of the Alzheimer type (DAT) are clinically classified into Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT) [1,2,10-12]. DAT are characterized by reduced content of acetylcholine and biochemical markers of presynaptic cholinergic function and pathological changes in the brain (neurofibrillary tangles and senile plagues). Blood contents of acetylcholine, its markers, and main component of senile plagues amyloid peptide Aβ$_{1-42}$ do not reflect their concentration in the brain because of the presence of these substances in peripheral tissues and the effect of the brain-blood barrier. The search for new biochemical criteria reflecting the pathophysiological state of the brain and suitable for clinical practice, early diagnostics of DAT, and differential diagnostics of various forms of DAT attracts much attention.

It was established that physiological disturbances in humans during various diseases could manifest in quantitative and qualitative changes in the contents of humoral immune factors, including autoantibodies (a-AB) to endogenous low-molecular-weight compounds [3,5]. A highly sensitive method of enzyme immunoassay was used to measure the content of a-AB to low-molecular-weight physiologically active substances in the blood from patients with various forms of DAT [4-7]. The concentration of a-AB to neurotransmitters (biogenic amines) and Aβ$_{1-42}$ was estimated. Biochemical differences were revealed in the ratio between the contents of a-AB to neurotransmitters (serotonin, histamine, norepinephrine, and epinephrine) and a-AB to Aβ$_{1-42}$ in patients with AD and SDAT [6]. Most neurotransmitters act as mediators of inflammation and modulate the state of vessels and blood coagulation system. It was interesting to estimate the content of a-AB

Department of Biochemical Assays, Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka; *Research-and-Methodical Center for Studies of Alzheimer's Disease and Associated Disorders, Research Center for Mental Health, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** mail nnlermon@mail.ru. Lermontova H. H.

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to some neuropeptides and proteins involved in inflammatory reactions and regulating vascular permeability and the state of the blood coagulation system, including bradykinin, vasopressin, thrombin, antithrombin III, α_2-macroglobulin (α_2-MG), and angiotensin II.

Here we measured the content of free a-AB to some peptides and ratio between the concentration of these antibodies and a-AB to $A\beta_{1-42}$ and its neurotoxic fragment $A\beta_{25-35}$ in the plasma from patients with SDAT and AD.

MATERIALS AND METHODS

Plasma samples for clinical biochemical assay were obtained from 27 patients with DAT (50-87 years). There were 14 patients with AD and 13 patients with SDAT. The diagnosis of DAT was made according to DSM-IV criteria [10] and NINCDS/ADRDA international diagnostic criteria for AD [14]. AD and SDAT were clinically differentiated using criteria developed by Russian scientists [1,2].

Synthetic $A\beta_{1-42}$ and its fragment $A\beta_{25-35}$ (Bachem), conjugated antibodies against human IgM labeled with horseradish peroxidase, and other reagents (Sigma) were used.

Synthesis of conjugated antigens and measurements of a-AB were performed as described previously for the isolation of conjugated antigens to peptides [4,7]. Conjugated antigens were synthesized in a solution of poly(4-nitrophenylacrylate) (3 mg, 0.02 mM) and 1 ml absolute dimethylformamide. Aβ was added to a final concentration of 0.001 mM. The concentration of a-AB was measured by solid-phase enzyme immunoassay. Blood plasma in various dilutions was placed in wells of a 96-well polystyrene plate (Nunc) pretreated with antigens. After incubation the plates were washed. Conjugated antibodies against human IgM labeled with horseradish peroxidase were added, maintained at 37°C for 1 h, and repeatedly washed. Immune complexes were detected using *o*-phenylenediamine as the substrate. The reaction was stopped by adding H_2SO_4 after 10-15 min. Optical density was measured on a Multiscan vertical-beam spectrophotometer at 492 nm.

The results were analyzed by Student's *t* test for samples with unequal variances.

RESULTS

a-AB to $A\beta_{1-42}$ and fragment $A\beta_{25-35}$ were revealed in plasma samples from all patients. The content of a-AB to fragment $A\beta_{5-35}$ was 28% higher than the concentration of a-AB to $A\beta_{1-42}$. The amount of a-AB to $A\beta_{1-42}$ and $A\beta_{25-35}$ in plasma samples from patients with AD insignificantly surpassed that in patients with SDAT (by 13%, Table 1). In patients with SDAT the content of a-AB to other test substances was higher than in patients with AD. The differences in the content of a-AB to bradykinin in patients with AD and SDAT were most pronounced (16.5%), but statistically insignificant.

For evaluation of a possible relationship between the increased content of a-AB to β-amyloids and low concentration of a-AB to other peptides in patients with AD (compared to patients with SDAT) we used ratios a-AB to peptides/Ab 1-42 (1) or a-AB to peptides/$A\beta_{25-35}$ (2) (Table 2). In patients with AD parameter 1 for bradykinin was much lower that in patients with SDAT. The mean values of parameter 2 for various peptides in patients with AD were lower than in patients with SDAT. These results suggest that a-AB to $A\beta_{25-35}$ are less selective than a-AB to $A\beta_{1-42}$, which is consistent with published data [6].

The relationship between the contents of a-AB to some biogenic amines [6] and the content of a-AB to bradykinin and $A\beta_{1-42}$ can result from the common pathological process. Bradykinin, histamine, and serotonin act as inflammatory transmitters and modulate vascular permeability and functions of the blood-brain

TABLE 1. Differences in the Contents of a-AB to $A\beta_{1-42}$, $A\beta_{25-35}$, and Other Peptides in Blood Samples from Patients with SDAT and AD (*M±SMEM*)

Parameter		SDAT	AD	AD-SDAT	AD-SDAT, % of AD	*p*
a-AB	to $A\beta_{1-42}$	0.423±0.024	0.486±0.036	0.063	13.0	0.16
	to $A\beta_{25-35}$	0.508±0.035	0.589±0.047	0.081	13.8	0.18
	to thrombin	0.432±0.019	0.395±0.031	-0.037	-9.37	0.33
	to antithrombin III	0.430±0.012	0.420±0.021	-0.01	-2.38	0.68
	to α_2-MG	0.381±0.020	0.368±0.028	-0.013	-3.53	0.71
	to angiotensin II	0.362±0.010	0.346±0.023	-0.016	-4.62	0.54
	to bradykinin	0.423±0.027	0.363±0.04	-0.06	-16.53	0.23
	to vasopressin	0.335±0.027	0.32±0.031	-0.015	-4.69	0.71

TABLE 2. Differences in the Ratio between the Contents of a-AB to Peptides and $A\beta_{1-42}$ (1) or $A\beta_{25-35}$ (2) in Plasma Samples from Patients with SDAT and AD (*M*±*SMEM*)

a-AB	Aβ	SDAT	AD	AD-SDAT	AD-SDAT, % of AD	*p*
To thrombin	1	1.052±0.068	0.855±0.083	-0.197	-23.04	0.14
	2	0.882±0.055	0.735±0.083	-0.147	-20.00	0.15
To antithrombin III	1	1.052±0.058	0.898±0.520	-0.154	-17.15	0.06
	2	0.880±0.044	0.771±0.069	-0.109	-14.14	0.19
To α_2-MG	1	0.917±0.044	0.797±0.075	-0.12	-15.06	0.18
	2	0.771±0.040	0.680±0.074	-0.091	-13.38	0.029
To angiotensin II	1	0.884±0.047	0.743±0.055	-0.141	-18.98	0.06
	2	0.739±0.036	0.638±0.062	-0.101	-15.83	0.17
To bradykinin	1	1.0594±0.1180	0.760±0.082	-0.299	-39.34	0.05*
	2	0.878±0.087	0.660±0.085	-0.218	-33.03	0.08
To vasopressin	1	0.827±0.083	0.667±0.052	-0.16	-23.99	0.12
	2	0.682±0.056	0.568±0.052	-0.114	-20.07	0.15

Note. *$p<0.05$, Student's *t* test for unequal samples.

barrier [8]. Published data show that inflammatory processes play a role in the pathogenesis of DAT. Antiinflammatory therapy of rheumatoid arthritis reduces the risk of DAT [13]. Patients with DAT are characterized by local inflammatory processes in the brain associated with neurodegeneration and causing the formation of senile plagues. Senile plagues contain not only aggregated $A\beta_{1-42}$, but also proteins of the complement system, cytokines, other inflammatory transmitters, thrombin, antithrombin III, and α_2-MG [9]. No differences were revealed in the ratio between the contents of a-AB to these peptides and a-AB to $A\beta_{1-42}$ in patients with AD and SDAT. The concentrations of a-AB to vasopressin, peptides, and angiotensin II affecting contraction of the vascular wall, as well as the ratio between their amount and contents of a-AB to $A\beta_{1-42}$, insignificantly differed in patients with various forms of DAT. The test peptides are not brain-specific and are present in the blood and other tissues. Changes in the content of a-AB to these peptides characterize the processes occurring not only in the brain. Some authors believe that the appearance of a-AB to $A\beta_{1-42}$ in the blood of patients with DAT is related to the impairment of the blood-brain barrier [15]. Little is known about changes in blood content of a-AB to physiologically active substances under normal and pathological conditions [3]. Therefore, it is difficult to interpret these results. Bradykinin is a potent regulator of blood-brain barrier permeability [8]. It can be hypothesized that the imbalance for ratio 1 for bradykinin and some biogenic amines [6] in patients with SDAT and AD reflect differences in bradykinin concentration and severity of disturbances in the blood-brain barrier permeability.

Blood samples from patients with SDAT and AD significantly differed by the ratio between the contents of a-AB to bradykinin and $A\beta_{1-42}$ (by 39%).

If we know this ratio, it is not necessary to measure the absolute content of a-AB in each plasma sample. Therefore, this method is well suitable for clinical practice. Further investigations of samples and blood from healthy donors of comparable age would elucidate the diagnostic and prognostic value of the proposed parameter.

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