Induction of Immune Complexes and Autoantibodies to Serotonin and Dopamine in Patients with Alzheimer's Disease

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Plasma concentration of immune complexes and antibodies to serotonin and dopamine were higher in patients with Alzheimer's disease compared to mentally healthy volunteers of the same age. Plasma concentrations of antibodies to serotonin and dopamine decreased with the increase in the duration of this disease.

Key Words: *Alzheimer's disease; immune complexes; antibodies; serotonin; dopamine*

The pathogenesis of Alzheimer's disease (AD), which is associated with neurodegenerative processes resulting in dementia, remains in many aspects unclear. It was hypothesized that immune mechanisms and especially local antigen—antibody reactions play an important role in the pathogenesis of AD. Antibodies (AB) against cholinergic structures that first undergo destruction in patients with AD attract much attention. AB to structural elements of brain tissue [9,13] and AB selectively binding to cholinergic neurons [5] were found in the plasma and spinal fluid from patients with AD. In rats active immunization with neurofibrillary protein from eel cholinergic neurons induced pathological changes similar to those observed during AD (*e.g.,* amnesia) [6].

Apart from damage to brain cholinergic structures, AD is accompanied by disturbances in other neurotransmitter systems, in particular serotonin- and dopaminergic systems [7,8,14]. AB to dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) probably play a role in the development of these disorders. Previous studies demonstrated the formation of auto-AB to 5-HT and DA in patients with Parkinson's disease (neurodegenerative disorder). These AB are involved in the pathogenesis of experimental parkinsonism [2,3].

Here we studied the formation of immune complexes (IC) and auto-AB to 5-HT (5-HT-AB) and DA at various stages of AD.

MATERIALS AND METHODS

We examined 16 patients with AD (women, 75-90 years) and 14 mentally healthy women of the same age. Psychiatric, neurological, and psychological examination was performed in all patients. Some patients were subjected to computerized axial tomography of the brain. Clinical diagnosis of AD was made by ADRDA and ICD-10 criteria (World Health Organization, 1994). The blood was taken once.

Plasma IC were assayed by precipitation in polyethylene glycol [4]. Experimental and control samples were measured on a SF-16 spectrophotometer at 450 nm. The results were expressed in extinction units (difference between experimental and control samples multiplied by 100).

AB to 5-HT and DA in the plasma were studied by ELISA on polystyrene plates treated with test antigens [1].

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Conjugates of 5-HT and DA with bovine serum albumin (BSA) synthesized by binding of neurotransmitters to diazotized protein (modified method [11]) served as test antigens. These antigens (200 µl) in a final concentration of 0.3 µg/well were placed in a Dynatech plate. After 1-day incubation at 37°C the plates were washed 4-5 times with physiological saline and 0.05% Tween 20. Test sera diluted 1:50 with in 0.05 M phosphate-saline buffer (pH 7.4) and 0.05% Tween 20 were incubated in the plates (200 µl per well) at 37°C for 1 h. The plates were washed and treated with secondary AB to human immunoglobulins labeled with horseradish peroxidase (dilution 1:2000). After 1-h incubation the plates were washed. The substrate mixture containing 10 µl 0.2 M $Na_2HPO_4 \times 2H_2O$, 10 ml 0.1 M citric acid, 8 mg *o*-phenylenediamine (Sigma), and 8 µl 33% H_2O_2 was added to wells. The incubation was performed in darkness for 1 h. The reaction was stopped with 6 N H_2SO_4. The amount of AB in each well was estimated by optical density on a Mini-reader device (Dynatech) at 495 nm and expressed in arb. units. The ratio of optical density of patient individual plasma to average optical density of the plasma from healthy volunteers was calculated. If this ratio surpassed 1.0 AB were present.

The results were analyzed by Student's *t* test (Statgraphics software).

RESULTS

In patients with AD plasma contents of IC and AB to 5-HT and DA were higher than in healthy volunteers (Table 1). The concentration of IC and 5-HT-AB in the plasma from patients 2-fold surpassed the control. AB to 5-HT and DA were found in 86.6 and 46.6% patients with AD and in 57.1 and 42.8% healthy volunteers, respectively. There are published data that AB to 5-HT and DA are present in healthy individuals and their content correlates with age [2,3]. However, plasma level of these AB in healthy individuals is low. In our experiments 5-HT-AB were found practically in all patients with AD, while the incidence of AB to DA did not differ from the control. For further analysis the patients were divided into 2 groups depending on the duration of AD: group 1 included patients with a history of AD less than 5 years and group 2 comprised individuals with more than 15-year history of AD (Table 2). In group 1 patients the concentration of AB to DA increased and considerably differed from that in group 2 patients (Table 2). AB to DA were revealed in 83.3 and 36.5% patients of groups 1 and 2, respectively. The content of 5-HT-AB was high in patients of both these groups. We found no considerable differences in the content of IC between group 1 and 2 patients.

TABLE 1. Content of IC and AB to Neurotransmitters in Patients with AD (*M*±*m*)

Parameter	Healthy volunteers (*n*=14)	Patients with AD (*n*=16)
IC, arb. units	18.8±1.6	32.9±3.9**
AB, arb. units		
to 5-HT	1.00±0.09	1.70±0.07*
to DA	1.00±0.09	1.30±0.09***

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to mentally healthy volunteers.

TABLE 2. Content of IC and AB to Neurotransmitters in Patients with Different Duration of AD (*M*±*m*)

Parameter	Group 1 (*n*=6)	Group 2 (*n*=10)
History of AD, years	4.5±0.5	18.1±1.6
IC, arb. units	26.8±3.5	39.2±5.0
AB, arb. units		
to 5-HT	1.72±0.10	1.73±0.10
to DA	1.6±0.2	1.16±0.06*

Note. *p<0.05 compared to group 1.

Thus we revealed intensive production of AB to 5-HT and DA in patients with AD, which attested to destabilization of serotonin- and dopaminergic systems. The content of IC also increased in these patients. High plasma content of IC in patients with AD was also reported by other investigators [11]. The concentrations of central [14] and peripheral 5-HT [10] sharply decrease in patients with AD. 5-HT deficiency is probably related to intensive production of the corresponding AB. Moreover, DA content in various brain structures also decreases in patients with AD [7,8]. These changes contribute to the development of depressive disorders most pronounced at the initial stages of the disease. Over the first 5 years of AD the concentration of AB to DA was high practically in all patients. In patients with more than 15-year history of AD the plasma level of AB to DA decreased and did not differ from the control. The role of AB to DA in the pathogenesis of AD requires further investigations.

REFERENCES

1. L. A. Basharova, V. A. Evseev, L. A. Vetrile, *et al*., *Byul. Eksp. Biol. Med*., **113**, No. 5, 469-471 (1993).
2. G. N. Kryzhanovskii, N. B. Man'kovskii, I. N. Karaban', *et al*., *Zh. Nevropatol. Psikhiatr*., No. 5, 21-26 (1994).
3. N. B. Man'kovskii, G. N. Kryzhanovskii, I. N. Karaban', *et al*., *Ibid*., No. 6, 7-11 (1993).
4. S. G. Osipov, N. I. Bakhov, V. N. Titov, *et al*., *Lab. Delo*, No. 6, 349-350 (1981).

5. J. Chapman, O. Bachar, A. D. Korzyn, *et al*., *J. Neurochem*., **51**, 479-489 (1988).
6. J. Chapman, G. Alroy, and Z. Zweiss, *Neuroscience*, **40**, 297-305 (1991).
7. S. Engelborns and P. P. De Deyn, *Acta Neurol. Belg*., **97**, 67-87 (1997).
8. P. Herregodts, M. Bruyland, and J. De Keyser, *J. Neurol. Sci*., **92**, 101-116 (1989).
9. F. Gaskin, B. S. Kingsley, S. M. Fu, *et al*., *J. Exp. Med*., **165**, 245-250 (1987).
10. A. M. Kumar, S. Senish, M. Kumar, *et al*., *Neuropsychobiology*, **32**, 9-12 (1995).
11. O. L. Lopez, B. S. Rabin, F. J. Huff, *et al*., *Stroke*, **23**, No. 8, 1078-1083 (1992).
12. B. Pescar and S. Spector, *Science*, **179**, 1340-1344 (1973).
13. K. Shott, H. Wormstall, M. Dietrich, *et al*., *Psychiatry Res*., **59**, 251-254 (1996).
14. G. M. Whitford, *Neuropsychobiology*, **15**, 133-142 (1986).