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Rapid communication

Decreased serum and red blood cell kynurenic acid levels in Alzheimer's disease

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Abstract

Kynurenine aminotransferases (KAT I and KAT II) are responsible for the transamination of kynurenine (KYN) to form kynurenic acid (KYNA), an excitatory amino acid receptor antagonist. Since these members of the kynurenine pathway (KP) are proposed to be involved in the pathogenesis of Alzheimer's dementia (AD), the activities of these enzymes and the levels of these metabolites were measured in the plasma and red blood cells (RBCs) of AD and control subjects together with the inheritance of the apolipoprotein (APOE) ε4 allele. KYNA levels were significantly decreased both in the plasma and in the RBCs in AD, but the levels of KYN and the activities of KAT I and KAT II remained unchanged. No association has been found with the possession of the ε4 allele. These findings indicate an altered peripheral KP in AD regardless of the APOE status of the probands.

Keywords: Alzheimer's dementia; Kynurenine pathway; Kynurenic acid; Kynurenine aminotransferase; Apolipoprotein E

An increasing body of evidence indicates that the kynurenine pathway (KP) of the tryptophan (TRP) catabolism is involved in Alzheimer's disease (AD) (Baran et al., 1999; Widner et al., 2000), the most common cause of dementia in the elderly. Kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN) are the main neuroactive compounds formed directly or indirectly from kynurenine (KYN). KYNA is an endogenous antagonist of the three ionotropic glutamate receptors (Perkins and Stone, 1982; Stone, 1993) and the α7 nicotinic acetylcholine receptor (Hilmas et al., 2001), and shows neuroprotective and anticonvulsive activities (Forster et al., 1984). KYNA is synthesized directly from KYN by enzymatic reaction using two kynurenine aminotransferases (KAT I and KAT II) (Guidetti et al., 1997). Furthermore, 3-HK is an oxidative stress generator and causes neuronal apoptosis (Okuda et al., 1998), while QUIN is an NMDA agonist and an endogenous excitotoxin (Chiarugi et al., 2001). Recent studies have revealed that QUIN neurotoxicity prevails mainly through lipid peroxidation and immune mediated processes, which is regarded as an important factor contributing to the proposed pathophysiology of AD and associated disorders (Rios and Santamaria, 1991; Guillemin and Brew, 2002; Zhu et al., 2004; Leonard and Myint, 2006).

Excitotoxicity, extensive oxidative stress and lipid peroxidation are all involved in the potential pathophysiology of AD (Ramassamy et al., 1999; Butterfield et al., 2002), An elevated KYNA content has been observed in the striatum of AD patients (Baran et al., 1999), which may in part be a compensatory response to QUIN neurotoxicity and other excitotoxic mechanisms, as it was found in other neurodegenerative disorders, like Huntington's disease (Sapko et al., 2006). On the other hand, the KYNA concentration in the cerebrospinal fluid was decreased (Heyes et al., 1992). Moreover, the increased plasma TRP/KYN quotient in AD (Widner et al., 2000) points to the importance of the KP in the neurodegenerative process. Additionally, the activity of indoleamine 2,3-dioxygenase (IDO), the rate-limiting

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enzyme of the KP, increases in AD, in parallel with the altered immune and oxidative stress responses (Heyes et al., 1992; Markesbery, 1997).

A large number of studies have demonstrated that the inheritance of the ε4 allele of the apolipoprotein E gene (APOE) is implicated in the pathogenesis of both the late-onset familial and the sporadic forms of AD (Poirier et al., 1993; Ramassamy et al., 1999). The extensive oxidative stress in correlation with the membrane lipid peroxidation in the brain of AD patients is influenced by the presence of the ε4 allele (Ramassamy et al., 1999; Butterfield et al., 2002). A higher level of lipoprotein oxidation in the CSF and in the plasma (Schippling et al., 2000), and an altered antioxidant status in the plasma (Bourdel-Marchasson et al., 2001; Rinaldi et al., 2004) and in the red blood cells (RBCs) (Serra et al., 1994; Rossi et al., 2002), have also been observed during the course of the disease.

Multiple lines of evidence demonstrate that the pathological changes in AD occur parallel in the brain and in the blood, but as yet no information is available regarding the neuroprotective part of the KP in the blood of AD patients. To shed light these questions, the KYN and KYNA contents, and the KAT I and KAT II activities were determined in the plasma and RBC of AD patients. The possibility of a relationship between the blood KP and APOE polymorphism was also examined.

1. Patients and methods

1.1. Subjects

Twenty-eight AD patients (6 males and 22 females; mean age 77 ± 6.3 years/S.D.) who met the NINCDS-ADRDA (McKhann et al., 1984) and DSM-IV criteria for probable AD, and 31 age-and sex-matched controls (CNTs) (10 males and 21 females; mean age 73 ± 8.3 years/S.D.), were enrolled in the study. The probable AD probands had the late-onset (over the age of 65 years) type of AD, with no family history of dementia. Their mini-mental state examination (MMSE) (Folstein et al., 1975) score was 21 ± 2.9 points (mean ± S.D.). The CNT group had a significantly ($p = 0.001$) higher MMSE score 28 ± 1.8 points (mean ± S.D.). All the subjects were Caucasian. The patients were drug-free or were not taking medication that could influence the dopaminergic system or the KP. Blood samples were obtained in accordance with the written informed consent procedures approved by the local ethics committee.

1.2. APOE genotype determination

DNA was extracted from white blood cells by the method of Davies (1986). The APOE genotyping was performed as described earlier (Kálmán et al., 1997).

1.3. Plasma and RBC KAT activity

The activities of the KATs were assayed by the modified method of Mason (1954), as described earlier (Hartai et al., 2005). Hemoglobin (Hb) and total plasma protein were used to normalize the results, which were expressed as pmol/mg Hb/h (for the RBCs) and pmol/mg protein/h (for the plasma).

1.4. Determination of KYN and KYNA

The endogenous KYN and KYNA concentrations were measured in the plasma and RBCs of the patients and CNTs on the basis of the method of Wu et al. (2000). The RBCs were hemolyzed with distilled water (1:1) and centrifuged (3500 rpm, 15 min, 24 °C), and 3200 μl of acetonitrile was added to 800 μl of hemolyzate to precipitate proteins. The aggregates were separated by centrifugation (3500 rpm, 10 min, 24 °C). The supernatant was placed into a thermostat at 50 °C, and concentrated under a nitrogen stream. The concentrates were dissolved in 500 μl of a mixture of distilled water/acetonitrile (1:1) and evaporated to dryness under nitrogen at 50 °C. Prior to analysis, the samples were dissolved in 150 μl of distilled water and the solution was centrifuged (12000 rpm, 10 min, 24 °C). Plasma was deproteinated with 8% perchloric acid (1:1) and centrifuged (12,000 rpm, 10 min, 24 °C). KYN and KYNA were quantitated by high-performance liquid chromatography (Agilent 1100 HPLC) with UV (365 nm, retention time: 2.3 min, KYN) and fluorescence detection (excitation: 344 nm, emission: 398 nm, retention time 6 min, KYNA). A 100 μl was applied onto a Hypersil 5 ODS HPLC column (150 mm × 4 mm, Thermo-Separation), and chromatographed isocratically at a flow rate of 1 ml/min with a mobile phase consisting of 0.2 M zinc acetate (pH 6.2) containing 5% acetonitrile. The KYN results were expressed as nmol/ml, and the KYNA results as pmol/ml.

1.5. Statistical analysis

All data were expressed as means ± S.E.M. In the KAT, KYN and KYNA studies one-way ANOVA followed by Fisher's LPSD test was used to determine the significance of differences between the groups. A level $p < 0.05$ was considered statistically significant. Correlation probes were used to establish the possible relation of the age of the patients with the KYN and KYNA levels, and the results are expressed with Pearson's coefficient. To test the possible KYN and KYNA concentration differences between the genders and the ε4 carrier and non-carrier patients, we used *t*-probes. Values are expressed as mean ± S.E.M., and *p* values were used to detect possible significance.

2. Results

Table 1 shows the distribution of different the APOE genotypes and the APOE allele frequencies of the different groups. APOE4 allele was overrepresented (23.5%) in the AD group as compared with the healthy CNTs (12.1%).

The activities of KAT I and KAT II and the concentrations of KYN and KYNA in the plasma and RBCs are depicted in Fig. 1. The plasma KYN levels (2.5 ± 0.1 μM versus 2.01 ± 0.2 μM), and the activities of KAT I (531.1 pmol/mg protein/h ± 10.7 versus 518.8 ± 15 pmol/mg protein/h) and KAT II (871 ± 15.2 pmol/mg protein/h versus 835.5 ± 19.5 pmol/mg protein/h) did not differ significantly. The KYNA level was significantly ($p < 0.05$) decreased in the AD plasma (15.8 ± 1.1 nM versus 23.13 ± 2.2 nM). There were no differences in the KYN level (8.1 ± 0.5 μM versus 9.3 ± 0.6 μM) or in the activities of KAT I

Table 1
Distributions of APOE genotypes and alleles

	Normal controls ($n = 29$)	Alzheimer's dementia ($n = 26$)
Genotypes		
2/2	0	0
2/3	6	1
3/3	16	13
3/4	7	11
4/4	0	0
2/4	0	1
Alleles		
E2	6 (10.3%)	2 (4%)
E3	45 (77.6%)	37 (72.5%)
E4	7 (12.1%)	12 (23.5%)*

* $p < 0.05$.