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.

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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 (KNA), 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 anticonvulsant 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...
enzymatic activity 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 e4 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 e4 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 RBCs 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 (8.1 ± 0.6); the MMSE score 28 (15.2 pmol/mg protein/h versus 19.5 pmol/mg protein/h) and KAT II (871 ± 2.2 nM) or in the activities of KAT I and the results are expressed with Pearson’s coefficient. To test the possible relation of the age of the patients with the KYN and KYNA levels, we used r-probes. Values are expressed as mean ± S.E.M., and p values were used to detect possible significance.

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 LSD 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 e4 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 KYNA level (8.1 ± 0.5 µM versus 9.3 ± 0.6 µM) or in the activities of KAT I

<table>
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<tr>
<th>Table 1 Distributions of APOE genotypes and alleles</th>
<th>Normal controls (n = 29)</th>
<th>Alzheimer’s dementia (n = 26)</th>
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<td>2 (4%)</td>
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<tr>
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</tr>
<tr>
<td>E4</td>
<td>7 (12.1%)</td>
<td>12 (23.5%) *</td>
</tr>
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</table>

* p < 0.05.
and KAT II (682.4 ± 53.1 pmol/mg Hb/h versus 625.7 ± 45.3 pmol/mg Hb/h) in the RBCs of the two groups. KYNA concentration was significantly (*p* < 0.05) lower in the RBCs of the AD probands (43.9 ± 5.9 nM versus 67.4 ± 8.6 nM).

No correlation was found with age, as revealed by the Pearson coefficients. There were no significant differences in KYN or KYNA contents between the genders, nor between the e4 allele carrier and non-carrier patients. A tendency to a decrease in the KYNA content of the RBCs was observed in the e4 carriers, but the effect was not significant (data are not presented).

### 3. Discussion

The interplay of the KYN metabolites between the brain and blood compartments during neurological diseases has come into the focus of interest (Heyes et al., 1994; Baran et al., 1995; Ilzecka et al., 2003) but to date only limited information is available. Our results demonstrate that the level of KYNA, the only known endogenous excitatory amino acid receptor antagonist, a neuroprotective and neuroinhibitory compound is significantly decreased in the plasma of AD patients. Heyes et al. (1992) similarly found a decreased KYNA content in the CSF of AD patients, while others observed selectively increased striatal KYNA levels, with only moderate increase in other brain areas (Baran et al., 1999), which may be a compensatory response to the hyperactive striato-frontal loop during the disease.

No correlation was found in our study between plasma KYNA levels and the age of the probands, and similar findings have been published by Kepplinger et al. (2005). Although KYNA levels were significantly lowered in the plasma of the AD patients, no alterations have been found neither in KYN levels nor in the KATs activities. Our results confirm earlier findings regarding the unaltered serum KYN level in AD (Widner et al., 2000). Further data support the idea of a disturbed KYN metabolism in AD, since lower TRP contents have been reported in the plasma and CSF of AD probands (Toghi et al., 1995; Fekkes et al., 1998) and an enhanced catabolism of TRP via the KP (Widner et al., 2000). It is tempting to speculate, therefore, that the altered KYNA metabolism in the blood of AD patients may influence the development of the dementia syndrome (Fuchs et al., 1990; Maes et al., 1994), but the relationship of the peripheral (blood) and brain KYNA metabolism and the pathomechanism of these AD-related processes remains to be elucidated.

To date, no results have been obtained regarding the neuroprotective part of the KP in the RBCs during AD, but changes may be predicted as oxidative processes and the TRP metabolism are associated in defined steps. Markers of oxidative and peroxidative damages are present not only in the AD brain and plasma, but also in the RBCs. Oxidative stress and mitotic abnormalities may also influence the erythropoiesis in the early stages of the neurodegenerative process, as various metabolic changes are observed in the RBCs during the early disease phase. The elevated activity of an antioxidant enzyme, copper-zinc superoxide dismutase (Cu2+, Zn2+ SOD) is an early
response in the RBCs of AD patients, but the elevation is not correlated with the presence of the APOE ε4 allele (Serra et al., 1994; Rossi et al., 2002). The glutathione redox status also indicates an increased oxidative damage in the RBCs of AD patients (Vina et al., 2004). Furthermore, circulating amyloid β-peptide (Aβ) causes membrane lipid peroxidation, and membrane lipid and protein destruction of the RBCs (Mattson et al., 1997). Further, the RBC Ca\(^{2+}\)-ATPase activity is decreased (Mattson et al., 1997), and the energy metabolism may therefore be impaired in these cells. KYNA formation is sensitive to the cellular energy status (Hodgkins and Schwarz, 1998). The KYNA level of the AD RBCs is decreased, which may be indicative of an impaired cellular energy metabolism. On the other hand, the levels of both KYN and KYNA are found to be in the same range, as we observed in the plasma. Neither the KYN content of the RBCs, nor the activities of the two examined KAT isoforms exhibited any difference. Although the reduced KYNA content of the RBCs does not have a direct effect on the CNS, further measurement of the neurotoxic metabolites of the KP in the RBCs would also be of interest.

Unfortunately there is as yet no explanation as to how our major findings, the decreased KYNA levels in the plasma and in the RBCs could contribute to the pathomechanism of AD. As regards the brain, recent studies demonstrate that the KP relates to oxidative stress in defined steps during the disease progression. QUIN, which is an endogenous NMDA agonist, a potent reactive oxygen species generator and a lipid peroxidation inducer (Rios and Santamaria, 1991; Santamaria et al., 2001), may be produced by the activated microglia, which enclose amyloid plaques. The concomitant presence of QUIN and Aβ can cause a rapid and neurotoxic glutamate release. The subsequent hyperactivity of the glutaminergic system leading to neuronal death and AD specific changes, like tau hyperphosphorylation, could be antagonised by selective NMDA antagonists, such as memantine with therapeutic relevance in AD (Li et al., 2004).

The QUIN-induced neurotoxic products of lipid peroxidation display elevated levels in the AD CSF and plasma (Vina et al., 2004; Selley et al., 2002). The oxidative and peroxidative processes may also relate to the KP in the case of altered IDO activity and QUIN production. In line with the increased activities of KAT I and KAT II, the KYNA level was elevated in the striatum of the AD brain, which may be a compensation of the hyperactive striato-frontal loop and also a neuroprotective response to the QUIN neurotoxicity (Baran et al., 1999). No data are yet available as concerns whether there are altered plasma contents of the neurotoxic KP products 3-HK and QUIN in AD. Recent data have demonstrated that in other neuroinflammatory or apoptotic conditions, such as brain edema or injury, the QUIN content is increased in the CSF and in the plasma (Smythe et al., 2003). The KYNA level, which can antagonize the effects of glutamate and QUIN, is decreased both in the CSF and in the plasma, which may point to a lower KYNA production from KYN. Although our results reveal unchanged activities of KAT I and KAT II in the plasma of the AD patients, the KYNA synthesis may deteriorate with the progression of the disease, as KYNA formation depends on the energy metabolism (Hodgkins and Schwarz, 1998), and a decreased energy metabolism has been demonstrated to be an early defect of AD.

ApoE4 acts as a risk factor in the neuroinflammation, Aβ deposition, neurofibrillary tangle formation and lipid dysfunction in AD (Refolo and Fillit, 2004). In addition, several data underline the correlations between ApoE4 and the oxidative damage, the extended lipid peroxidation and the altered activities of the antioxidant enzymes in the brain and in the periphery (Ramassamy et al., 1999; Shea et al., 2002). In our study, we tested whether a correlation exists between the inheritance of the ε4 allele with the KYN and KYNA levels of the RBCs. The APOE allele frequencies reported here are in the same range as published earlier (Kálmán et al., 1997). The mean KYN levels demonstrate no difference between the ε4 carrier and non-carrier subjects. A tendency to a decrease in the KYNA level was detected in the ε4 carriers, but this was not significant. As no significant differences were found for any of the examined parameters of the KP, we conclude that it is unlikely that the ApoE status of the AD patients would modify the neuroprotective part of the KP.

One important limitation of our study is that the neurotoxic kynurenic metabolites (3-HK, QUIN) were not measured. We cannot exclude the possibility therefore that the KP is shifted from the neuroprotective to the neurotoxic products in the periphery.

Another limitation of our work that we cannot explain why the decreased plasma KYNA levels are not associated with altered KAT activities. Since KYNA is not known to be metabolized further, one possible explanation could be the increased renal excretion. This explanation could be excluded, since opposite findings, even elevated plasma KYNA levels have been reported in patients with chronic renal failure (Pawlak et al., 2002). Furthermore, neither our AD probands nor any data from the literature indicate that AD is associated with renal disturbances.

Overall, our findings indicate a disturbed peripheral KP in AD, but the ApoE ε4 carrier status is unlikely to exert effects on the KYNA synthesis and the KYN and KYNA levels in the plasma and RBCs.

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References


