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Kynurenine metabolism in plasma and in red blood cells in Parkinson's disease

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Abstract

Substantial evidence indicates that neuroactive kynurenine metabolites play a role in the normal physiology of the human brain, and are involved in the pathology of neurodegenerative disorders such as Parkinson's disease (PD). A sidearm product of the pathway, kynurenic acid (KYNA), which is synthesized by the irreversible transamination of kynurenine (KYN) by kynurenine aminotransferases (KAT I and KAT II), is an excitatory amino acid receptor antagonist. In the present study we measured the level of KYNA and the activities of the biosynthetic enzyme isoforms KAT I and KAT II in the plasma and in the erythrocytes (RBC) of 19 PD patients and 17 age-matched controls. The KAT I and KAT II activities were significantly lower in the plasma of PD patients, followed by a tendency to a decrease in plasma KYNA. An elevated KYNA level correlated with a significant increase in KAT II activity in the RBC of PD patients. These data support the contribution of an altered KYNA metabolism in the RBC to the pathogenesis of PD. The increased activity of KAT II in correlation with the elevated KYNA level in the RBC may mediate a consecutive protective response against excitatory neurotoxic effects.
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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder with an unknown etiology. The selective death of dopaminergic neurones in the substantia nigra pars compacta (SNc) results in degeneration of the nigrostriatal pathway. Clinical symptoms first occur after 50% cell loss in the SNc [1,2]. A substantial body of evidence points to the roles of oxidative stress and excitotoxicity in the pathogenesis of the disease.

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Although PD is a CNS disease, neurochemical changes can be observed in other organs. Decreased levels of vitamin C [3] and GSH [4,5] have been reported in the plasma and lower GSH peroxidase [6] and superoxide dismutase (SOD) levels [7,8] have been measured in the RBC in PD. These data suggest alterations can be detected in the plasma and RBC in PD.

3-Hydroxykynurenine (3-HK) is a neuroactive metabolite of the kynurenine pathway which can cause apoptotic cell death [9]. Another product of the kynurenine metabolism is quinolinic acid (QUIN), which is an *N*-methyl-D-aspartate (NMDA) receptor agonist and an endogenous excitotoxin [9,10]. Kynurenic acid (KYNA) is a sidearm product of the pathway. It exerts broad-spectrum antagonism on the three ionotropic excitatory

amino acid receptors [11,12] and non-competitive antagonism at α7 nicotinic acetylcholine receptors [13,14]. It has been demonstrated that KYNA may act as a potent neuroprotective, neuroinhibitory and anticonvulsive compound [15,16]. The two enzyme isoforms that are primarily responsible for the synthesis of KYNA are kynurenine aminotransferase I and II (KAT I and KAT II). The enzymes have different pH optima (pH 9.6 for KAT I and pH 7.4 for KAT II) [17,18], and exhibit different catalytic characteristics.

An altered kynurenine metabolism and KYNA levels have been reported in certain brain regions in PD [19,20]. The KYNA concentration was reduced in the frontal cortex and putamen while 3-HK was significantly increased in the putamen and SNc in patients with PD [19]. Beal et al. found no alterations in the KYNA content in the precentral gyrus (A4) and in the caudate of the PD patients [20]. Altered plasma KYNA levels have been detected in several neurological diseases such as amyotrophic lateral sclerosis (ALS) [21] and complex partial seizure [22]. As far as we are aware, the kynurenine metabolism in the blood in PD has not been reported previously. There are as yet no data on changes in the KYNA level and activities of the biosynthetic enzymes KAT I and KAT II in the plasma in PD. Furthermore, no studies on KYNA and KAT isoforms in the RBC during neurological or psychiatric illnesses have been reported.

The aim of this study was to determine the activities of KAT I and KAT II, and to measure the neuroprotective KYNA concentrations in the plasma and RBC in order to establish whether this system is involved in the pathogenesis of PD.

2. Materials and methods

2.1. Patients

This study was conducted on 19 patients with PD (11 males and 8 females, aged 58–76 years, mean age 69.1 ± 10.4 years, mean ± S.D.). The diagnosis of PD was proved by the cardinal clinical signs (resting tremor, rigor and bradykinesis) and by the sustained good responds to L-DOPA. The patients were on unchanged L-DOPA medication for at least 3 months prior to the study. None of the subject took dopamine agonists. The average UPDRS III score was 32.5, and the Hoehn-Yahr stage was III. Seventeen age-matched volunteers (8 males and 9 females, aged 55–74 years, mean age: 67.8 ± 12.4 years, mean ± S.D.) served as a control group. They had never received any dopaminergic medication. The blood samples were taken between 9 and 11 am, separated immediately and kept at −80 °C until measurements. None of the subjects were on a special diet.

The study was approved by the Human Investigation Review Board of the University and informed consent was obtained from each patient participating in the study.

2.2. Materials

Kynurenine sulphate, kynurenic acid, pyridoxal-5′-phosphate, 2-oxoglutarate and zinc acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was from Scharlau (Madrid, Spain). Other chemicals were from different commercial suppliers and were the purest available or of analytical grade.

2.3. Kynurenine aminotransferase activity

The activities of the KATs were assayed by the modified method of Mason [23]. After withdrawal the RBC and plasma were frozen separately and stored at −80 °C until they were measured. The RBC were haemolysed with distilled water. The reaction mixture contained 100 μl of haemolysed RBC or plasma sample, 10 mM 2-oxoglutarate, 40 μM pyridoxal-5′-phosphate, 1 mM KYN in 1/15 M phosphate buffer, pH 9.6 for KAT I measurements and pH 7.4 for KAT II measurements. No KYN was added to the blank. After incubation for 1 h at 37 °C, the reaction was terminated by the addition of boric acid. The enzyme activity was detected spectrophotometrically at 333 nm. Haemoglobin and total plasma protein were used to normalize the results, which were expressed as pmol/mg Hb/h (for RBC) and pmol/mg protein/h (for plasma samples).

2.4. Determination of kynurenic acid

The endogenous KYNA concentration was measured in the RBC and plasma of the patients and controls on the basis of the method of Baran et al. [24]. The RBC were haemolysed with distilled water. Perchloric acid was added to plasma or haemolysed RBC to precipitate proteins. The samples were centrifuged at 6000 rpm for 10 min. The supernatant was added to 600 mg of Dowex 50W cation-exchange resin treated with 5 ml of 0.1 M HCl 3 times. The samples were vortexed for 3 min then HCl was added. After vortexing, the supernatant was discarded and the KYNA was eluted in 40 ml of water. The eluates were immediately frozen to −80 °C and lyophilized under vacuum. Prior to analysis, samples were dissolved in 240 μl of distilled water. KYNA was quantitated by high-performance liquid chromatography (Agilent 1100 HPLC, Waldbroon, Germany) with fluorescence detection according to the method of Swartz et al. [25]. Briefly, samples were applied onto a Hypersil 5 ODS HPLC column (150 × 4 mm, Thermo-Separation, Bellefonte, PA, USA), and chromatographed isocratically at a flow rate of 1 ml/min with a mobile phase consisting of 0.2 M zinc acetate and 5% acetonitrile, pH 6.2. KYNA was detected by a fluorescence detector (excitation: 344 nm and emission: 398 nm).

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