Elevated levels of Harman and Norharman in cerebrospinal fluid of Parkinsonian patients

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Summary. Death of dopaminergic neurons in Parkinson’s disease (PD) may partially be caused by synthesis and accumulation of endogenous and exogenous toxins. Because of structural similarity to MPTP, β-carbolines, like norharman and harman, have been proposed as putative neurotoxins. In vivo they may easily be formed by cyclization of indoleamines with e.g. aldehydes. For further elucidation of the role of β-carbolines in neurodegenerative disorders harman and norharman levels in cerebrospinal fluid (CSF) were measured in 14 patients with PD and compared to an age- and sex-matched control group (n = 14). CSF levels of norharman and harman in PD were significantly higher compared to controls. These results may suggest a possible role of harman and norharman or its N-methylated carbolinium ions in the pathophysiological processes initiating PD. However the origin of increased levels of these β-carbolines remains unclear. On the one hand one may speculate, that unknown metabolic processes induce the increased synthesis of harman and norharman in PD. On the other hand a possible impact of exogenous sources may also be possible.

Keywords: Parkinson’s disease, harman, norharman, cerebrospinal fluid

Introduction

The potent neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces cell death in dopaminergic neurons (Sayre et al., 1990). β-carbolines, like harman and norharman, show structural resemblance to MPTP and its ion MPP⁺ (Neafsay et al., 1989; Sayre et al., 1990). In vivo they may easily be formed by cyclization of indoleamines with e.g. aldehydes (Neafsay et al., 1989; Sayre et al., 1990). Therefore several studies were initiated to elucidate the potential neurotoxicity of these compounds. After application of N-methylated β-carbolinium ions to rats by intracerebral infusion only a weak neurotoxicity was found because of limited uptake into
dopaminergic neurons (Sayre et al., 1990). In contrast N-methylated β-carbolinium ions, like 2-methyl-norharman, induce dopamine depletions, large lesions and gliosis after injection in the substantia nigra of rats (Neafsay et al., 1989). The best candidates for a MPPt like neurotoxicity of β-carbolinium ions are the 2,9-dimethylated forms (Collins et al., 1992). Therefore increased levels of toxic N-methylated carbolines may be one initiating factor in idiopathic parkinsonism (Matsubara et al., 1993). The underlying pathophysiological processes remain unclear up to now. 2-methylated β-carbolines may inhibit NADH-coenzyme Q reductase (complex 1) of the electron transport chain within mitochondria, thereby leading to a fall in ATP production and thus initiating cell death (Albores et al., 1990). Such a decrease in the activity of complex I has consistently been found in the brain, especially in homogenates of the substantia nigra, but also in platelets, muscle and lymphocytes of Parkinsonian patients (Schulz and Flint Beal, 1994). Aim of the present study was to determine the harman and norharman CSF levels in treated and de novo Parkinsonian patients and to compare them to age- and sex-matched controls.

**Material and methods**

CSF samples taken from 14 idiopathic Parkinsonian subjects (7 male, 7 female; mean age 60 ± 8.7, range 40–71; mean duration of PD 3.98 ± 4.15 (SD), range 0.3–15 years; n = 1 Hoehn Yahr Scale I, n = 4 Hoehn Yahr Scale II, n = 7 Hoehn Yahr Scale III, n = 1 Hoehn Yahr Scale IV) were analyzed. 7 patients received conventional Parkinsonian pharmacotherapy including l-dopa/benserazid or carbidopa preparations (n = 7), selegiline (n = 6), bromocriptine (n = 6), lisuride (n = 1) and 7 were previously untreated “de novo” Parkinson patients. The control group was age- and sex-matched and consisted of 14 patients without peripheral neurologic disorders (7 male, 7 female; mean age 60 ± 9.37, range 43–74). Patients with metabolic disturbances or other central neurological diseases beneath Parkinson’s disease and subjects with clinical or biochemical signs of alcoholism, heroinism or depression were excluded.

**Sample collection**

After 10 hours patients’ fasting and resting in bed lumbar puncture was performed between 8 a.m. and 9 a.m with the patient in the lateral decubitus position and before arising from bed. Patients gave informed consent. CSF was collected directly from the needle in three different test tubes for (1) cell count and cell morphology, glucose, chloride, total proteins, isoelectric focusing and electrophoresis (8ml) and (2) measurement of norharman and harman. Sample 2 was immediately frozen and stored at −80°C. Blood-tinged CSF was discarded, samples with a cell count greater than 5 WBC/ml, or with abnormal protein, glucose or chloride content were not used. Time period between freezing and work up of CSF samples for estimation of β-carbolines was no longer than three months. Measurement of norharman and harman levels in CSF was performed by high-performance liquid chromatography (HPLC) (Rommelspacher et al., 1991a,b).

**Statistical analysis**

Comparison of norharman and harman CSF levels of Parkinsonian patients and age- and sex matched controls were performed by the two tailed Mann-Whitney U-test. For correlation linear regression was used.