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The urate and xanthine concentrations in the cerebrospinal fluid in patients with vascular dementia of the Binswanger type, Alzheimer type dementia, and Parkinson's disease

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Summary. We determined the urate and xanthine concentrations in the cerebrospinal fluid (CSF) in patients with vascular dementia of the Binswanger type (VDBT), Alzheimer type dementia (ATD), and Parkinson's disease (PD). We found that the urate concentration was significantly increased in VDBT patients, but significantly decreased in ATD patients compared with controls. The ratio of the concentrations of uric acid (U_{CSF}) to xanthine (X_{CSF}) in the CSF (U_{CSF}/X_{CSF}) had a significant correlation with the ratio of the U_{CSF} to the urate concentration in serum (U_{serum}) (U_{CSF}/U_{serum}) in ATD and PD, whereas U_{CSF}/U_{serum} increased independently of U_{CSF}/X_{CSF} in VDBT. We concluded that the significant increase in the urate concentration in VDBT is mainly due to an impairment of the blood-brain barrier (BBB), and its significant reduction in ATD may reflect impaired brain metabolism.

Keywords: Urate, xanthine, vascular dementia of the Binswanger type, Alzheimer type dementia, Parkinson's disease, cerebrospinal fluid

Introduction

Purine nucleotides participate in a number of important biochemical processes as the monomeric units of nucleic acids, as components of energy-rich end products of most energy-releasing pathways, as the coenzymes, secondary messengers, purinergic mechanisms, and others. They are synthesized de novo, or from the degradation products of nucleic acids, and are degradated into oxypurines (hypoxanthine, xanthine) and urate. Therefore, changes in the urate and oxypurine concentrations in the cerebrospinal fluid (CSF) may provide clues for alterations of nucleotide metabolism in brain tissues. Previous studies have reported that urate and oxypurines increased in the CSF of patients with acute ischemic brain diseases (Hällgren et al., 1983), alcoholic withdrawal states (Carlsson and Dencker, 1973), and senile

dementia of Alzheimer type (SDAT) and multi-infarct dementia (Degrell and Niklasson, 1988). However, a major problem concerning the urate and oxypurine concentrations is their origins and interpretation of results obtained. It has been thought that urate in the CSF was exclusively derived from plasma, because xanthine oxidase, which catabolizes hypoxanthine to xanthine, and xanthine to urate, was absent or only present in very small amounts in the mammalian brain (Al-Khalidi and Chaglassian, 1965), and because the urate concentration in serum is about 20 times higher than in the CSF (Farstad et al., 1965). However, later studies have suggested that xanthine oxidase is present in the brain (Aoki et al., 1984; Betz, 1985). In contrast, the xanthine concentration in the CSF of healthy individuals is about 5 times higher than that in serum (Niklasson, 1983), is not profoundly influenced by the blood-brain barrier (BBB), and may reflect the intracellular nucleotide pool in the brain.

We studied the urate and xanthine concentrations in the CSF from patients with vascular dementia of the Binswanger type (VDBT), Alzheimer type dementia (ATD), and Parkinson's disease (PD), compared with patients with multiple infarcts with preserved intelligence, and controls. To explore potential changes in brain uncleoide metabolism, and in the BBB in such diseases, we evaluated the correlation between the ratio of the urate (U_{CSF}) and xanthine (X_{CSF}) concentrations in the CSF (U_{CSF}/X_{CSF}) and the ratio of the urate concentrations in the CSF (U_{CSF}) and serum (U_{serum}) (U_{CSF}/U_{serum}), assuming that these ratios are closely related in the steady-state.

Materials and methods

Subjects

Subjects were 15 patients with VDBT (69 ± 6 years) (mean ± S.D.), 10 patients with ATD (68 ± 8 years), 11 patients with parkinson's disease (67 ± 6 years), 6 patients with multi-infarcts with normal intelligence (70 ± 6 years), and 14 controls (68 ± 6 years). The duration of the diseases was 3.8 ± 4.1 years for VDBT, 3.9 ± 2.1 years for ATD, 2.4 ± 0.8 years for PD, and 3.8 ± 2.1 years for multi-infarcts unassociated with dementia. Control CSF samples were obtained from neurologically normal patients who underwent hemorrhoidectomy at the time of lumbar anesthesia before surgery. All patients were admitted to the hospital, placed on the same standard diet, and were drug-free for at least 2 weeks. Informed consent was obtained from all patients prior to this study.

Diagnostic criteria

The diagnosis of ATD and VDBT was made according to DSM-III-R (American Psychiatric Association, 1987), Hachinski's Ischemic Score (Hachinski et al., 1975), the criteria of the NINCDS-ADRDA Work Group (McKhann et al., 1984), and CT and MRI findings. All patients with VDBT had a diffuse and extensive low density area on CT scans, and diffuse high intensity area on T_2-weighted MRI in the cerebral white matter (leukoaraiosis; Hachinski et al., 1987), not associated with infarcts greater than 3 cm in diameter. We strictly excluded patients having vascular lesions for the diagnosis of ATD. However, we were not able to exclude the possibility that some of our patients diagnosed as having VDBT may actually have had mixed dementia (VDBT

plus ATD). The intellectual ability was assessed by the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). The MMSE scores were 12.6 ± 4.1 for VDBT patients, and 11.9 ± 4.4 for ATD patients. The diagnosis of PD was made based upon clinical history, symptoms, and CT and MRI studies, excluding vascular parkinsonism, progressive supranuclear palsy, striato-nigral degeneration, olivopontocerebellar atrophy, and other diseases presenting with parkinsonism.

CSF analysis

Lumbar CSF (5 ml) was obtained with the patients in the lateral decubitus position between 9.00 and 10.00 h after overnight bed-rest and fasting. CSF was placed on ice immediately after lumbar puncture and stored at −80°C.

The concentrations of urate and xanthine were determined by injection of 80 µl volumes of the CSF into a reverse phase C_{18} column (Neuro Column, Niko Bioscience, Tokyo). Sample analysis was performed using high-performance liquid chromatography (HPLC) with a 16-sensor Neurochemical analyser (ESA Inc., Bedford, MA) (Matson et al., 1984) as reported previously (Tohgi et al., 1993). The mobile phase A consisted of 0.1 M sodium phosphate and 10 mg/l sodium dodecyl sulfate at pH 3.35; the mobile phase B consisted of 50% methanol/10.1 M sodium phosphate and 50 mg/l sodium dodecyl sulfate at pH 3.45. Both were obtained directly from ESA as final reagents. The 16 serial electrodes were set in an incremental 60 mV array from 0 mV to 900 mV. The column and electrodes were maintained at 37°C. Standards were obtained from the Sigma Chemical Company. The detection limit was 20 pg/ml and the recovery rate was nearly 100%. Interassay variances were less than 5%. The concentration of urate in serum was obtained in a routine laboratory examination by the uricase-peroxidase method.

Statistics

The inter-group differences of mean values were analysed by one-way analysis of variance. The correlation coefficients were calculated as the Pearson product-moment correlation.

Results

We found that the urate concentration in the CSF was increased significantly in VDBT patients ($p < 0.001$), but decreased significantly in ATD patients ($p < 0.05$) compared with controls (Table 1). The mean xanthine concentrations in the CSF were lower in all the patient groups compared with controls, although the differences were significant only for PD patients

Table 1. The urate and xanthine concentrations in the CSF and the urate concentration in serum (µmol/l). *VDBT* vascular dementia of the Binswanger type; *ATD* Alzheimer type dementia

	n	Urate in CSF	Xanthine in CSF	Urate in serum
Controls	14	5.52 ± 1.26	3.08 ± 0.81	303 ± 70
Multi-infarct patients	6	6.57 ± 2.63	2.30 ± 0.70	226 ± 86
VDBT	15	8.05 ± 1.31**	2.72 ± 1.15	283 ± 91
ATD	10	4.39 ± 1.14*	2.55 ± 0.53	262 ± 88
Parkinson's disease	11	5.00 ± 1.68	2.48 ± 0.48*	277 ± 48

*$p < 0.05$, **$p < 0.001$ versus controls

($p < 0.05$). The urate and xanthine concentrations in the CSF had no significant correlation with MMSE.

There were non-significant trends of positive correlation between the urate concentrations in the CSF and in serum in VDBT patients ($r = 0.47$, $p = 0.08$), and between the xanthine concentration in the CSF and the urate concentration in blood in ATD patients ($r = 0.56$, $p = 0.09$) (Table 1). Otherwise, we did not find significant correlations between the urate concentration in the CSF (U_{CSF}), the xanthine concentration in the CSF (X_{CSF}), and the urate concentration in serum (U_{serum}). Therefore, the U_{CSF}/X_{CSF} and U_{CSF}/U_{serum} ratios vary among individuals, suggesting that the urate concentration in the CSF does not depend upon the conversion from xanthine to urate alone, nor upon the transport capability across the BBB against a concentration gradient alone. We found a non-significant trend only of positive correlation between U_{CSF}/X_{CSF} and U_{CSF}/U_{serum} in controls (r 04 0.41, $p = 0.19$). However, we found a significant correlation between the U_{CSF}/X_{CSF} and U_{CSF}/U_{serum} ratios in ATD ($r = 0.71$, $p < 0.05$), PD ($r = 0.66$, $p < 0.05$), and multi-infarct patients ($r = 0.83$, $p < 0.05$) (Fig. 1), suggesting that the conversion of xanthine to urate and the elimination of urate from the CSF are closely related in these groups. In contrast, VDBT patients had no such correlation between U_{CSF}/X_{CSF} and U_{CSF}/U_{serum} ($r = 0.03$, $p = 0.92$), and the U_{CSF}/U_{serum} ratio increased regardless of the U_{CSF}/X_{CSF}, indicating an insufficient elimination of urate from the CSF compared with the influx of urate from the brain and blood to the CSF.

Discussion

Our main findings were: (1) that the urate concentration in the CSF was significantly reduced in ATD, whereas it was significantly increased in VDBT compared with controls; (2) that the xanthine concentration in the CSF and the urate concentration in serum displayed a tendency to decrease in all patient groups, and the reduction in the xanthine concentration in the CSF was significant in PD; and (3) that the urate concentration in the CSF relative to that in serum had a significant positive correlation with the ratio of the concentrations of urate to xanthine in the CSF in ATD, PD, and multi-infarct patients, but not in VDBT patients.

Our results that the urate concentration increased significantly in VDBT, but decreased significantly in ATD could not be attributed to the urate concentrations in serum, which were not different among the patient groups, and had no significant correlation with that in the CSF. It seems, therefore, that the urate concentration in the CSF is not profoundly influenced by that in serum at least within the normal range, and unless the BBB is severely altered. The observed relationship between the urate concentration in the CSF relative to that in serum (U_{CSF}/U_{serum}) and the ratio of the urate to xanthine concentrations in the CSF (U_{CSF}/X_{CSF}) in patient groups of ATD, PD, and multi-infarcts suggests that the conversion from xanthine to urate and elimination of urate from the CSF are closely related in these patients.

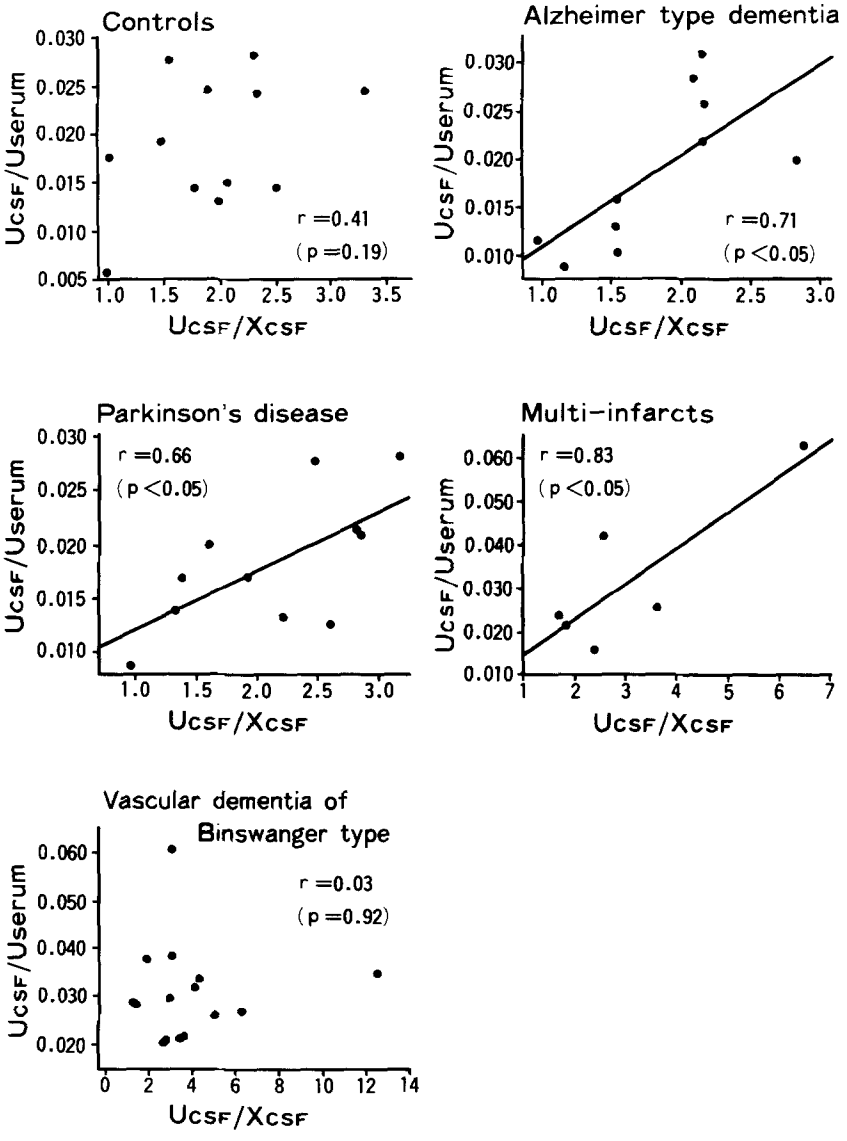

Fig. 1. The correlations between the ratio for the urate (U_{CSF}) to xanthine (X_{CSF}) concentration in the CSF (U_{CSF}/X_{CSF}), and the ratio of the U_{CSF} to the urate concentration in serum (U_{serum}) (U_{CSF}/U_{serum}). Note: differences in scales for patient groups are due to different ranges of values

However, we did not find a clear significant correlation between the U_{CSF}/U_{serum} and U_{CSF}/X_{CSF} rations in the controls as we did in ATD and PD patients. This may be because we did not perform CT scans for the controls, and some of them might have had asymptomatic small infarcts, whereas the diagnosis of ATD and PD was made excluding patients having vascular lesions on CT scans.

The observed reduction in the urate concentration in the CSF of ATD patients is opposite to the findings of Degrell and Niklasson (1988) in SDAT

patients. This discrepancy may be partly explained by the fact that their SDAT patients (mean age 77 years) were much older than our ATD patients (mean age 68 years), and that the urate and xanthine concentrations increase with advancing age (Tohgi et al., 1993), probably due to decreased blood flow and metabolism in some of the elderly subjects. The reduction in the urate concentration in ATD patients may indicate reduced cerebral metabolism, because xanthine oxidase utilizes NAD^+ as an electron acceptor and is inhibited by NADH (Kaminski and Jezewska, 1981). Positron emission tomography studies have shown reduced glucose metabolism in ATD brains (Benson et al., 1983). A similar reduction in the urate concentration has been demonstrated in Huntingtonian brains (Beal et al., 1991). The observed non-significant decrease of the xanthine levels in ATD patients may also suggest a reduction in the brain nucleotide pool due to degeneration and metabolic depression of cerebral tissues. Similarly, the significant reduction in the xanthine concentration in PD patients suggest an impaired cerebtal nucleotide metabolism. Studies of the cerebral metabolic rate for glucose in PD patients have shown either no consistent difference compared with controls (Rougemont et al., 1984), or a moderate generalized reduction (Kuhl et al., 1984).

An increase in the urate concentration has been demonstrated in the CSF of patients with ischemic brain disease (Hällgren et al., 1983), and also in experimental ischemia in rat (Kanemitsu et al., 1988), which was inhibited by allopurinol (Nihei et al., 1989). The observed increase in the urate concentration in VDBT patients may be largely related to a disturbance of the BBB, because the urate concentration in the CSF had a non-significant trend to have a positive correlation with that in serum, and because the U_{CSF}/U_{serum} ratio was increased independently of the U_{CSF}/X_{CSF} ratio. Previous studies have also demonstrated a higher CSF/serum albumin ratio in vascular dementia compared with controls (Erkinjuntti and Sulkava, 1991; Wallin and Blennow, 1991; Leonardi et al., 1985), suggesting impaired integrity of the BBB. Although urate reacts with free radicals as an anti-oxidant, the reaction in turn generates the urate anion free radical which can be scavenged by ascorbate (Maples et al., 1988). Such a free radical may exert adverse effects on the brain. It has been reported that ascorbate was consumed in experimental cerebral ischemia in cat (Flamm et al., 1978). The lack of an increase in the xanthine concentration in the CSF precludes the possibility that the increased urate concentration in the CSF may reflect an increased accumulation of nucleotide degradation products in the brain.

We do not know the reasons for the non-significant reduction in the xanthine concentration in spite of the significant increase in the urate concentration in the CSF of patients with multi-infarcts as well as VDBT. Although it is known that the xanthine concentration increases in the acute phase of stroke (Hällgren et al., 1983), it may decrease in the brains with reduced metabolism after degenerative precesses associated with ischemia have progressed.

In conclusion, the urate concentration in the CSF was significantly reduced in ATD suggesting impaired cerebral metabolism, whereas it was significantly increased in VDBT, mainly reflecting impaired integrity of the BBB.

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