ELSEVIER

Journal of Chromatography A, 680 (1994) 243–246

JOURNAL OF CHROMATOGRAPHY A

Capillary zone electrophoretic determination of organic acids in cerebrospinal fluid from patients with central nervous system diseases

Atsushi Hiraoka*[,a], Junichiro Akai[a], Itaru Tominaga[b], Munekazu Hattori[b], Hideki Sasaki[c], Teruyo Arato[d]

[a]*Department of Mental Health, Kyorin University School of Health Sciences, Hachioji, Tokyo 192, Japan*
[b]*Department of Neuropsychiatry, Chiba National Hospital, Chiba, Chiba 260, Japan*
[c]*Nihon Milipore, Waters Chromatography Division, Shinagawa-ku, Tokyo 140, Japan*
[d]*Department of Biochemistry, Kyorin University School of Medicine, Mitaka, Tokyo 181, Japan*

Abstract

Organic acids in cerebrospinal fluid (CSF) from patients with various central nervous system (CNS) diseases were determined by capillary zone electrophoresis (CZE). Under one of the two sets of conditions employed, several anionic components of CSF were separated into corresponding peaks on the electropherograms and determined. The other conditions employed were also useful in measurement of the lactate contents in CSF. The CSF levels of lactate and pyruvate and the ratios of lactate to pyruvate were elevated in patients with cerebral infarction and bacterial meningitis, whereas CSF ascorbate was reduced mainly in inflammatory disorders of the CNS. The results showed that CZE can become a powerful tool in the biochemical diagnosis of CNS diseases.

1. Introduction

Cerebrospinal fluids (CSF) has been examined biochemically for the diagnosis of central nervous system (CNS) diseases. It is known from earlier studies that lactate (LA) is the most abundant among the organic acid components of CSF and that its concentrations are increased in cerebral infarction [1] and bacterial meningitis but not in viral meningitis [2–6]. Such changes in the CSF levels of LA were virtually independent of the serum levels of LA [7], and the CSF contents of LA and pyruvate (PA) were positively correlated with each other [8], indicating that the concentration ratios of LA to PA were greater in CSF with higher LA levels [9]. It is therefore generally accepted that increased LA concentrations in CSF, which are generally accompanied by elevated CSF LA/PA values, can reflect the pathological conditions of the CNS in association with tissue acidosis and/or anaerobic glycolysis caused by ischaemia or bacterial activity. The concentrations of these compounds in CSF have conventionally been measured mainly by high-performance liquid chromatography (HPLC) or enzymatic methods. However, capillary zone electrophoresis (CZE) is a simple and valuable system for the determination of a variety of ionized substances, such as low-molecular-mass cations [10], drugs [11–14], vitamins [15]

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00362-D

and sugar derivatives [16]. In this work, CZE was applied to the determination of CSF organic acids, including LA and PA, as an aid in neurological diagnosis.

2. Experimental

2.1. Subjects and samples

Forty CSF samples were taken by lumbar puncture from five cerebral infarction, two bacterial meningitis, two viral meningitis, one Guillain–Barré syndrome, three Parkinson's disease, two senile dementia, three multiple sclerosis, six epileptic, two schizophrenic, three depressive illness, five neurosis and six other micellaneous neuropsychiatric disorder patients (17 males and 23 females, 18–79 years old). All the samples were stored at −20°C for up to 2 weeks until analysed. Before the analyses, CSF samples were deproteinized by centrifuging ultrafiltration at 740 *g* for 30 min with Centricon-10 miniconcentrators (Grace Japan), and 20-μl portions each from the ultrafiltrates thus prepared were pipetted into sample vials of the two CZE instruments.

2.2. Conditions of CZE analyses

Two conditions, A and B, were established employing a Waters Quanta-4000 unit equipped with a negative power supply and a Beckman P/ACE 2000 unit, respectively. Under conditions A, samples to be examined, which were obtained from 28 patients, including two acute-phase cerebral infarction, one chronic-phase cerebral infarction, two bacterial meningitis, two viral meningitis, one Guillaine–Barré syndrome and two shizophrenic, were injected by hydrostatic loading for 60 s into a fused-silica capillary (60 cm × 75 μm I.D.), and separation was done at 20 kV using 50 m*M* sodium tetraborate (pH 9.2) containing 2.5% tetradecyltrimethylammonium bromide (TTAB) (Nihon Millipore) as the electrolyte. UV detection was performed at 185 nm and the data were processed with a Waters 825 Data Station. Under conditions B, samples from twelve patients, including two cerebral infarction, were injected by hydrostatic loading for 20 s into a capillary (50 cm × 75 μm I.D.), and separation was done at 20 kV using 100 m*M* borate buffer (pH 8.3) as the electrolyte. UV detection was performed at 200 nm. Under both conditions, the capillaries were conditioned at the start of each analysis by purging with 0.1 *M* potassium hydroxide solution for 1 min, deionized water for 1 min and the electrolytes for 2 min.

3. Results and discussion

A typical electropherogram obtained under conditions A is illustrated in Fig. 1. Several peaks in addition to a large peak of chloride (Cl) were detected on the electropherograms, and peaks with the migration times (t_M) of *ca.* 4.9, 5.2, 5.5, 5.8, 5.9, 6.3, 6.6 and 6.8 min were identified as oxalate, fumarate, inorganic phosphate, acetate, PA, LA, glutamate (Glu) and ascorbate (AsA), respectively, by mixed analyses with the authentic samples. Among these anions, oxalate, acetate and LA, and also two major inorganic anions (Cl and phosphate), were detected in all 28 samples examined, although fumarate, PA, Glu and AsA were detected only in 6, 22, 9 and 23 samples, respectively, out of the 28. In the experiments using standard samples with the concentrations of 1.56–800 μg/ml, the detection limits of these seven organic acids (including Glu) were in the range 1.56–3.12 μg/ml, and linear increases in the peak areas were

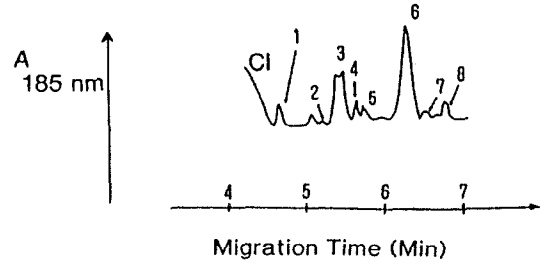

Fig. 1. Typical electropherogram obtained under conditions A. Cl = chloride; 1 = oxalate; 2 = fumarate; 3 = inorganic phosphate; 4 = acetate; 5 = pyruvate; 6 = lactate; 7 = glutamate; 8 = ascorbate.

also confirmed in the concentration ranges 0–800 μg/ml (LA) and 0–100 μg/ml (others). Therefore, the levels of these compounds in the CSF examined could be determined from the ratios of peak areas against the 100 μg/ml standard samples.

The mean ± standard deviation (S.D.) CSF LA concentration was 147.4 ± 83.7 μg/ml (n = 28), and the highest and lowest values were 475 and 66 μg/ml, respectively. Evaluated LA levels above 180 μg/ml were found only in CSF from two acute-phase cerebral infarction patients and two bacterial meningitis patients. All the other 24 CSF samples, including those from a chronic-phase cerebral infarction patient and two viral meningitis patients, had LA concentrations within the range 66–173 μg/ml. The mean ±S.D. CSF PA level (the value for six samples in which this compound was not detected were taken as 0 μg/ml) was 4.1 ± 2.8 μg/ml (n = 28), and the highest value (12.2 μg/ml) observed in a bacterial meningitis patient was associated with the highest level of CSF LA mentioned above.

In 22 CSF samples in which PA was detected, a positive correlation (r = 0.72) was observed between the levels of PA and LA. The mean ±S.D. LA/PA value in above-described four CSF samples with elevated LA levels of 180–475 μg/ml was 36.8 ± 4.4 μg/ml (n = 4), which was significantly greater ($p < 0.05$) than that in other samples (28.0 ± 3.3 μg/ml, n = 18) with non-raised LA levels of 66–173 μg/ml and detectable amounts of PA.

All of these trends in pathological changes in the CSF levels of LA and Pa agreed with those reported by earlier workers who examined CSF by HPLC or enzymatic methods [1–9] (see Introduction). The mean ±S.D. CSF AsA concentration (a value obtained in the same manner as for PA) was 5.2 ± 2.9 μg/ml (n = 28). This compound was not detected in five CSF samples, three of which were taken from patients with inflammatory disorders of the CNS (bacteral meningitis, viral meningitis and Guillain–Barré syndrome). This trend was in agreement with that revealed by our previous work employing HPLC, in which we demonstrated that the relative AsA concentrations in CSF to serum were reduced in various CNS diseases, especially in such inflammatory disorders [17].

Glu was detected in nine CSF samples out of the 28 examined. Kim *et al.* [18] reported that Glu decreased in CSF of patients with schizophrenia, although in CSF samples from two schizophrenic patients treated in this study (see Section 2.1) this compound was detected, suggesting that there is no decrease in its level in CSF in schizophrenia. Virtually no diagnostic value for other CSF organic acids detected by this system was found owing to the small amounts present.

A typical electropherogram obtained under conditions B is illustrated in Fig. 2. Several peaks were detected on the electropherograms, and those with $t_M \approx 4.5$ and 8.8 min were identified as glutamine (Gln) and LA, respectively. Under these conditions, peaks of organic acids other than LA, which were detected and identified under conditions A (Fig. 1), were not so separated well enough for these CSF minor organic acids to be determined. However, the concentrations of LA in twelve CSF samples examined were measured, and the results were essentially the same as those for the other 28 samples examined under conditions A, as the mean ±S.D. CSF LA level in these twelve samples was 112.9 ± 58.6 μg/ml (n = 12) and levels above 180 μg/ml (the highest was 224 μg/ml) were detected only in two cerebral infarction patients.

It was also noteworthy that only under these conditions peaks of Gln, which is a neutral amino acid, and LA, which is an organic acid, appeared on the same electropherograms. The cause of this difference in the electropherogram

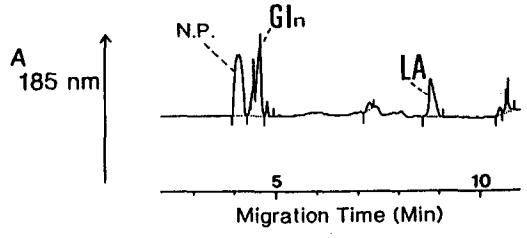

Fig. 2. Typical electropherogram obtained under conditions B. N.P. = neutral peak; Gln = glutamine; LA = lactate.

patterns obtained under conditions A and B was assumed to be as follows. Under conditions B, which do not use TTAB and a negative power supply, the capillary has a cathodic electroosmotic flow (EOF), which carries Gln, having little charge and low mobility at this pH (8.3), to the cathode. On the other hand, LA with a negative charge has a migration potential to the anode, but organic acids have a low net mobility. Therefore, LA was also carried to the cathode with the strong EOF. The CZE analyses under conditions B, which is a capillary isotachophoresis system previously developed by us [19], can become a useful tool for the determination of Gln in CSF, an increase in which is an important marker of hepatic encephalitis [20].

The present results indicate that CZE is a powerful tool for the determination of organic acids in CSF as an aid in the biochemical diagnosis of CNS disease. In analyses under conditions A, a high resolution of peaks which was achieved with the use of a negative power supply, and addition of TTAB to the electrolyte enabled us not only to determine LA, the major organic component of CSF, but also to detect changes in the levels of some other CSF minor organic acids in association with pathological conditions of the CNS. However, in analyses under conditions B, evaluated levels of LA in CSF can be clearly detected. This type of examination of CSF is very valuable for estimating the brain damage in ischaemia of cerebral infarction [1] and also to distinguish between bacterial meningitis and viral meningitis [2–6].

References

[1] R. Zupping, A.E. Kaasick and E. Ravdam, *Arch. Neurol.*, 23 (1971) 33.
[2] G. Controni, W.J. Rodriguez and J.M. Hicks, *J. Pediatr.*, 91 (1977).
[3] I. Brook, K.S. Bricknell and G.D. Overturf, *J. Infect. Dis.*, 137 (1977) 379.
[4] R.A. Koromorowski, S.G. Farmer and G.A. Hanson, *J. Clin. Microbiol.*, 8 (1978) 89.
[5] E. D'Souza, B.K. Mandel and J. Hooper, *Lancet*, 2 (1987) 579.
[6] A. Hiraoka, I. Miura, M. Hattori, I. Tominaga, K. Kushida and M. Maeda, *Biol. Pharm. Bull.*, 17 (1994) 1.
[7] J.B. Posner and F. Plum, *Arch. Neurol.*, 16 (1967) 492.
[8] B.K. Seijo, *Brian Energy Metabolism*, Wiley, New York, 1978, p. 607.
[9] R.A. Fishman, *Cerebrospinal Fluid in Diseases of the Nervous System*, W.B. Saunders, Philadelphia, 1980, p. 231.
[10] A. Weston, P.B. Brownm, P. Jandick, W.R. Jones and A.L. Heckenberg, *J. Chromatogr.*, 593 (1992) 289.
[11] S.K. Yeo, H.K. Lee and S.F.Y. Li, *J. Chromatogr.*, 585 (1991) 133.
[12] M.T. Ackermans, J.L. Beckers, F.M. Everets, H. Hoogland and M.J.H. Thomassen, *J. Chromatogr.*, 596 (1992) 101.
[13] U. Seita, P.J. Oefner, S. Nathakarnkitkkol, M. Popp and G.K. Brown, *Electrophoresis*, 13 (1992) 35.
[14] G.L. Chee and T.S.M. Wan, *J. Chromatogr.*, 612 (1993) 172.
[15] E. Kenndler, C. Schwer and D. Kaniansky, *J. Chromatogr.*, 508 (1990) 203.
[16] S. Honda, S. Iwase, A. Makino and S. Fujisawa, *Anal. Chem.*, 176 (1989) 72.
[17] A. Hiraoka, I. Miura, M. Satô, M. Hattori and I. Tominaga, *J. Anal. Bio-Sci.*, 13 (1990) 176.
[18] J.S. Kim, H.H. Kornhuber, W. Schmid-Burk and B. Holzmüller, *Neurosci. Lett.*, 20 (1980) 379.
[19] A. Hiraoka, I. Miura, M. Hattori and I. Tominaga, *Clin. Biochem.*, 22 (1989) 293.
[20] B.T. Hourani, E.M. Hamlin and T.B. Raymond, *Arch. Intern. Med.*, 177 (1991) 1033.