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Capillary-zone electrophoretic determination of lactate in cerebrospinal fluid from patients with central nervous system diseases

Atsushi Hiraoka,* Isao Miura,* Itaru Tominaga,**
Munekazu Hattori** and Hideki Sasaki***

SUMMARY

A rapid and microscale analysis system for organic acids in cerebrospinal fluid (CSF) was established using capillary-zone electrophoresis (CZE). On the electropherograms obtained under the conditions employed, a peak corresponding to lactate (LA) was separated from those of other anionic components in CSF, such as oxalate, inorganic phosphate, acetate and ascorbate, and the LA concentrations of CSF were determined quantitatively. The results showed that the LA levels in CSF were elevated beyond the upper normal limit of 200 $\mu\text{g/ml}$ in some of patients with cerebral infarction and bacterial meningitis, but not in other central nervous system (CNS) diseases including cerebrovascular diseases other than cerebral infarction and viral meningitis. It was, therefore, suggested that the present CZE system is useful as an aid in the biochemical diagnosis of CNS diseases.

Key words: capillary-zone electrophoresis, cerebrospinal fluid, organic acid, lactate, central nervous system diseases.

INTRODUCTION

Cerebrospinal fluid (CSF) has been analyzed for making the diagnosis of neurological diseases, since the levels of its constituents change under various pathological conditions of the central nervous system (CNS). Earlier studies on organic acids in CSF revealed that lactate (LA) was the most abundant and that the LA concentrations increased beyond the upper normal limit of 200 $\mu\text{g/ml}$ in cerebral infarction¹⁾ and in bacterial and fungal meningitis but not in viral meningitis.²⁻⁶⁾ Such changes in the levels of LA in CSF were virtually independent of the changes in the serum

LA level.⁷⁾ Therefore, it has been generally accepted that increases in the CSF LA contents reflect the pathological conditions of the CNS in association with tissue acidosis and/or anaerobic glycolysis.⁸⁾ Thus, measurement of the LA concentration in CSF is valuable as an aid in the biochemical diagnosis of the CNS diseases, especially in making distinction between bacterial meningitis and viral meningitis and also in evaluating the brain tissue damage in cerebral infarction. At present, LA in CSF is determined by high-performance liquid chromatography (HPLC) or the enzymatic method. Capillary-zone electrophoresis (CZE) is a rapid and microscale technique

* 平岡 厚, 三浦勇夫; Department of Mental Health, Kyorin University School of Health Sciences.

** 富永 格, 服部宗和; Department of Neuropsychiatry, Chiba National Hospital.

*** 佐々木秀輝; Waters Chromatography Division, Nihon Millipore Co.

Correspondence address: Atsushi Hiraoka, Kyorin University School of Health Sciences, 476 Miyashita-cho, Hachioji, Tokyo 192, Japan.

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for the analyses of a variety of ionic substances. One of the authors recently succeeded in separating and determining organic acids in food samples by CZE.⁹⁾ The authors attempted to apply CZE to the analyses of organic acid components of CSF, particularly LA as an aid in the biochemical diagnosis of the CNS diseases.

MATERIALS AND METHODS

Subjects

Fifty-eight CSF samples were taken by lumbar puncture from 50 patients (23 males and 27 females, 18-76 years old) with a variety of neuropsychiatric diseases, as summarized in Table 1. In order to prevent the *in vitro* formation of LA in CSF after sampling, all the CSF samples were immediately frozen and stored at -20°C for up to one week until examined, leaving the rest required for routine laboratory tests in the hospital.

Analytical conditions

CZE was carried out employing a Waters Quanta-4000 CZE apparatus equipped with a fused silica column ($50\ \mu\text{m} \times 60\ \text{cm}$) and a Negative Power Supply. Before analyse, 0.1-ml portions of CSF samples were centrifuged in Centricon-10

miniconcentrators (Grace Company) at $740 \times g$ for 15 min, to afford the protein free ultrafiltrates (ca. $30\text{--}40\ \mu\text{l}$). $10\text{-}\mu\text{l}$ portions of the ultrafiltrates thus obtained were placed in sample vials, and then injection into the capillary was performed by the hydrostatic method ($20\ \text{s}$; $1.28 \times 20 = 25.6\ \text{nl}$). Separation was achieved at 15 kV for 10 min using 120 mM sodium borate containing 5% (v/v) Nice-Pak (Nihon Milipore) as the electrolyte, and separated peaks were detected by the direct monitoring at 185 nm. The identifications of oxalate, inorganic phosphate, acetate, LA and ascorbate were carried out by mixed analyses with the authentic samples. The quantitative interpretation of the data were performed on the basis of a Waters Maxima 805 Data Station.

Determination of CSF LA by HPLC

Twenty μl each of the ultrafiltrates of 10 CSF samples were injected to HPLC under the separatory conditions for analyses of organic acids as described previously.¹⁰⁾

RESULTS AND DISCUSSION

One of the typical electropherograms of organic acids in cerebrospinal fluid obtained under the conditions employed was shown in Fig. 1A. As shown in Fig. 1A, several peaks with the migration time (M_t) of ca. $3.04 \pm 0.08\ \text{min}$ ($n=54$), $3.47 \pm 0.11\ \text{min}$ ($n=58$), $3.72 \pm 0.10\ \text{min}$ ($n=33$), $3.97 \pm 0.09\ \text{min}$ ($n=58$) and $4.28 \pm 0.06\ \text{min}$ ($n=41$) in addition to a large peak for chloride ion (M_t : 0.8-2.2 min) were detected on the electropherograms. They were identified as oxalate, inorganic phosphate, acetate, LA and ascorbate, respectively. Among them, peaks for inorganic phosphate and LA, as well as one for chloride, were detected on the electropherograms of all the 58 CSF samples examined. Oxalate, acetate and ascorbate were observed only in 54, 33 and 41 CSF samples, respectively.

With the authentic samples detection limits of these compounds are found to be ranged between 3.13 and 6.25 $\mu\text{g/ml}$, and linearity of the standard curves obtained using the authentic samples was

Table 1. Diseases and numbers of patients and samples used.

Diseases	Numbers of	
	Samples	Patients
Cerebral infarction	6	5
Other vascular diseases of CNS	7	7
Bacterial meningitis	5	2
Viral meningitis	4	2
Other inflammatory disorders of the CNS	3	2
Multiple sclerosis	4	3
Other degenerative disorders of the CNS	5	5
Epilepsy	5	5
Schizophrenia	2	2
Depressive illness	3	3
Neurosis	6	6
Other neurological disorders	8	8
Total	58	50

Fig. 1. Capillary-zone electrophoresis of cerebrospinal fluid (A: with normal lactate level, B: elevated lactate level).

The vertical and horizontal lines indicate absorption at 185 nm (mV) and migration time (min), respectively. Peak identification: a, chloride; b, oxalate; c, inorganic phosphate; d, unidentified; e, acetate; f, lactate; g, unidentified; h, ascorbate.

confirmed within the concentration range of 0–800 $\mu\text{g/ml}$. The LA concentrations of CSF samples examined were calculated from the ratio of peak area using the standard sample of 100 $\mu\text{g/ml}$ LA. The mean value and standard deviation were 171.4 ± 124.6 $\mu\text{g/ml}$ (Mean $\pm$ S.D., $n=58$, C.V.: 72.7%) and the highest and lowest values were 74 and 685 $\mu\text{g/ml}$, respectively. The CSF LA concentrations obtained by CZE were close to those determined by HPLC; $r=0.93$, $n=10$, $Y=1.03X+4.5$ (X : CZE, Y : HPLC). The elevated LA levels in CSF beyond the upper normal limit of 200 $\mu\text{g/ml}^{2-6)}$ were observed only in 3 samples from 3 cerebral infarction patients and 4 samples from 2 bacterial meningitis patients. All the samples from patients with other CNS diseases including cerebrovascular diseases other than cerebral infarction and viral meningitis exhibited the normal LA levels of below 200 $\mu\text{g/ml}$. One of the electropherograms showing an elevated LA level is shown in Fig. 1B. It was further revealed that in a patient with bacterial

Table 2. Lactate and protein levels and cell counts in CSF during the course of hospitalization on 2 patients with bacterial meningitis.

Cases	Days after admission	Lactate ($\mu\text{g/ml}$)	Protein (mg/dl)	Cells*
A	0	685	330	L 940 N 138/3
	14	298	140	L 105 N 33/3
	28	214	60	L 35 N 10/3
B	0	360	125	L 240 N 92/3
	28	193	45	L 16 N 5/3

* Numbers of lymphocytes (L) and nucleocytes (N) /3 in 1 mm³.

meningitis, from whom CSF samples were taken 3 times during the hospital course, the CSF LA concentrations were reduced in response to improvement as the CSF levels of total proteins and cell counts decreased (Table 2).

Among organic acids other than LA in CSF, pyruvate, which was detected in 33 of 58 samples examined, was increased above 10 $\mu\text{g/ml}$ (the highest; 25 $\mu\text{g/ml}$) only in the above mentioned 7 samples from 3 patients with cerebral infarction and 2 patients with bacterial meningitis having the elevated CSF LA concentrations above 200 $\mu\text{g/ml}$ (up to 685 $\mu\text{g/ml}$). In a sample from a patient with bacterial meningitis, the highest pyruvate level was accompanied by the highest LA concentration. On the other hand, a detectable amount of ascorbic acid (up to 18 $\mu\text{g/ml}$) was present in 41 of 58 samples examined. This compound (17 samples, in total) was absent mainly in inflammatory disorders in brain and/or meninges (8 samples). These results supported an earlier observations that the CSF concentrations of LA and pyruvate were positively correlated with each other,¹¹⁾ and also agreed with the results of our previous study with reduced CSF ascorbate levels in various CNS diseases, especially in the inflammatory disorders.¹⁰⁾ Furthermore, as shown in Fig. 1B, two unidentified peaks with the M_t of ca. 3.6 min and 4.1 min, respectively, were also detected on the electropherograms of some CSF samples with the elevated PA and pyruvate levels. However, these minor anionic components in CSF did not allow us to determine their CSF con-

centrations by the present CZE system. Oxalate was detected in the bulk of CSF examined (93% = 54/58) giving a mean value of $6.4 \pm 3.1 \mu\text{g/ml}$ ($n=58$; a value with 4 samples in which this compound was not detected was taken as $0 \mu\text{g/ml}$) and the highest value was $15 \mu\text{g/ml}$. The CSF oxalate level was not different under pathological conditions. In order to elucidate changes in the levels of CSF organic acids other than LA by CZE, sensitivity must be increased.

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