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Sodium dodecylsulfate capillary gel electrophoretic measurement of the concentration ratios of albumin and α_2-macroglobulin in cerebrospinal fluid and serum of patients with neurological disorders

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Abstract

Sodium dodecylsulfate capillary gel electrophoresis (SDS-CGE) was applied to measure the concentration ratios of albumin (Alb) and α_2-macroglobulin (αMG) in the cerebrospinal fluid (CSF) and concurrent serum samples from patients with various neurological disorders. The values of the αMG index in individual patients were calculated on the basis of the peak area ratios of Alb and an αMG subunit on the CSF and serum electropherograms. The αMG index value thus obtained was most prominently raised in patients with inflammatory diseases of the brain and/or meninges, suggesting that the function of the blood–brain barrier (BBB) was disturbed under the pathological conditions in the central nervous system. The measurement of the concentration ratios of Alb and αMG in CSF and the concurrent serum samples by the present SDS-CGE system seems to be useful as an aid in the biochemical examination of the BBB function in patients with neurological disorders.

Keywords: Albumin; Macroglobulin; Globulins; Proteins

1. Introduction

The blood–brain barrier (BBB) is a mechanism which is present in the choroid plexus of the brain and restricts the entrance of macromolecular substances from the blood plasma into the central nervous system (CNS). Under human physiological conditions with the normal BBB function, the total protein (TP) content in the cerebrospinal fluid (CSF) is only 0.2–0.5% of that in the blood plasma, and among the proteins in the CSF, the components with a higher molecular mass (M_r) generally have a lower concentration relative to those in the blood plasma. However, during various pathological conditions with a disturbed BBB function, the CSF TP level was often elevated beyond the upper normal limit of 40 mg/dl, and the macromolecular plasma proteins with a M_r value higher than 160 000, such as α_2-macroglobulin (αMG), low-density lipo proteins, the haptoglobin polymer, immunoglobulin M (IgM), etc., increase in the CSF [1,2]. Examination of the BBB function in patients with neurological disorders has been performed by measurement of the [αMG]CSF / [αMG]serum · [Alb]serum / [Alb]CSF (αMG index) [3] (=[Alb]serum/[αMG]serum· [αMG]CSF/[Alb]CSF) values, since both albumin (Alb) (M_r: 68 000) and αMG (720 000, 4 subunits) are major components among the plasma proteins

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and not produced in the CNS. The conventional methodology used is the determination of Alb and αMG in the CSF and the concurrent serum by enzyme-linked immunosorbent assay (ELISA) [4], which requires complicated procedures and expensive reagents. As an aid in the biochemical examination of the BBB function using the αMG index value as a marker, we applied sodium dodecylsulfate capillary gel electrophoresis (SDS-CGE), which has been used as a rapid and simple technique for the analysis of proteins with different M_r values [5–8] to analyze Alb and αMG in the CSF and the concurrent sera. It is also expected that the concentration ratio of Alb and αMG in the samples can be obtained from the area ratio of the corresponding peaks on the electropherograms (note that the determination of the absolute contents of these body fluid proteins is not necessary if the above-described transformed formula in parentheses is employed). In this study, the αMG index values thus calculated are evaluated from the viewpoint of clinical biochemistry of neurological disorders.

2. Experimental

2.1. Subjects

CSF samples were taken by lumbar puncture from 51 patients (23 males and 28 females, 18–74-years-old) with the following various neurological disorders: cerebral infarction (7 cases), cerebrovascular disease-type dementia (3), meningitis and meningoencephalitis (4), Altzheimer's disease and senile dementia of the Altzheimer type (4), Parkinson's disease (4), amyotrophic lateral sclerosis (2), other neurodegenerative diseases (3), multiple sclerosis (5), epilepsy (4), peripheral neuropathy with no organic damage in the CNS (5), schizopherenia (3), manic-depressive illness (4) and neurosis and tension headache (3). The concurent sera were prepared from the blood specimens obtained at the same time from their arm veins. Informed consent was obtained from all the patients. All the CSF and serum samples were stored at −20°C, leaving the rest required for routine laboratory tests in the hospital, such as the determination of the TP level in both the CSF and serum, the counting of the cell numbers in the CSF, etc.

2.2. Chemicals

All the reagents were of analytical grade. The standard samples of Alb, αMG and other human body fluid proteins were purchased from Sigma (St. Louis, MO, USA). An eCAP SDS-200 kit was purchased from Beckman (Fullerton, CA, USA).

2.3. Pre-treatments of samples

The CSF samples with a TP level (based on the routine laboratory test in the hospital) of 11–125 mg/dl were quantitatively concentrated by centrifugal ultrafiltration at 740 *g* for 10–30 min using Centricon-30 miniconcentrators (Amicon, Japan). The concentration ratio of each sample was obtained from the sample volumes before and after centrifuging. The final TP level in the CSF concentrates ranged between ca. 620 and 970 mg/dl (recovery ratio was considered as 100%), which was ca. 9–13% of that of the concurrent serum samples, respectively. The individual serum was diluted using deionized water, so that the final TP content was equal to that of the concentrated sample of the corresponding CSF. Samples to be injected were then prepared based on the protocol of the Beckman eCAP SDS 200 kit (Beckman). Briefly, a mixture of the concentrated CSF or diluted serum (50 μl), the sample buffer (120 m*M* trishydroxymethylaminomethane buffer containing 1% SDS, pH 6.6) (50 μl), Orange G as the front marker (5 μl) and 2-mercaptoethanol (2.5 μl) was heated at 100°C for 5 min and then cooled to the room temperature.

2.4. Analytical conditions

As the instumentation, a Beckman PACE 2000 capillary electrophoresis analyzer was employed. A fused-silica capillary as a kit content (47 cm×100 μm), of which the inner surface was coated with linear polyacrylamide, was attached to the apparatus in the reversed polarity mode. A constant volume of

each sample, prepared as above, was injected at 3.4 kPa for 60 s into the capillary filled with the SDS-containing gel buffer as a kit content, of which the chemical structure has not been published by Beckman. The separation was done at 14.1 kV and 20°C for 30 min and the detector was operated at 214 nm. These are generally the same conditions as previously employed for the analyses of the CSF low-M_r proteins [9] and M_r microheterogeneity in the CSF β-trace protein (βTP) [10], except that the separation time was lengthened from 25 to 30 min. Identification of the peaks on the electropherograms was performed by mixed analyses with the standard samples of the corresponding proteins. A mixture of the marker proteins with M_r values of 29 000 (carbonic anhydrase), 43 000 (ovalbumin), 66 000 (bovine serum albumin), 97 000 (phosphorylase B), 116 000 (β-galactosidase) and 205 000 (myosin) as a kit content was also co-electrophoresed. The data for measurement of the peak area ratio of Alb and αMG on the electropherograms were analyzed using System Gold computer software (Beckman).

2.5. Capillary-zone electrophoresis (CZE) of the CSF and serum proteins

This was carried out in order to detect and identify proteins which increased in the CSF of patients who exhibited elevation of the TP level in spite of the fact that the αMG index values were low. A constant volume of each concentrated CSF or diluted serum without the denaturating treatments, as shown in Section 2.3, was injected by pressure (0.5 p.s.i.; 1p.s.i.=6894.76 Pa) for 10 s into another fused-silica capillary (57 cm×75 μm) for the CZE attached in the normal polarity mode to the same apparatus as described in Section 2.4. The separation was run using 100 m*M* borate buffer (pH 10.0) at 15 kV and 20°C for 12 min and monitored at 214 nm. These are generally the same as in the case of the determination of βTP in the CSF low-M_r protein fraction [11], except that no internal standard was used and that the injection time was shortened from 40 to 10 s. βTP was identified due to the disappearance of its peak by passage of the intact CSF through a column with an immunoaffinity resin to human βTP [12] before the concentration step (Section 2.4).

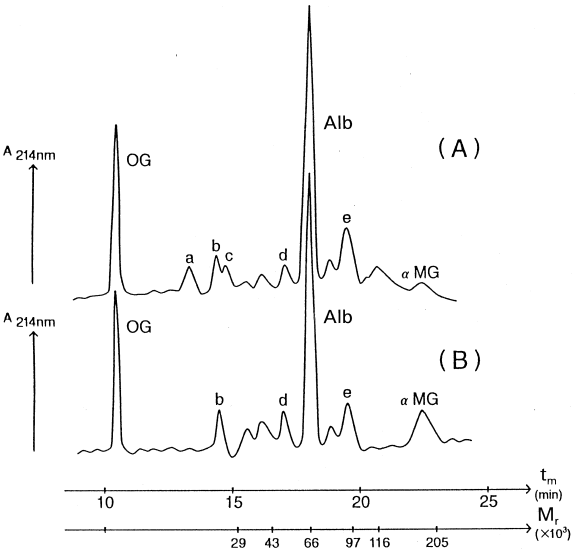

Fig. 1. Typical SDS-CGE electropherograms of the CSF (A) and serum (B) proteins. OG=Orange G as the front marker (see Section 2.3). Peaks: (a) transthyretin, (b) IgG light chain, (c) β-trace protein, (d) IgG heavy chain. Alb=albumin, e=transferrin, αMG=α_2-macroglobulin.

3. Results and Discussion

Typical electropherograms of the CSF and serum proteins are shown in Fig. 1. As shown in Fig. 1, the peak patterns of the CSF (Fig. 1A) and serum (Fig. 1B) resembled each other. Indeed, at least seven peaks were commonly observed. Among them the most abundant peak with a migration time (t_m) of ca. 18 min and a small one with a t_m value of ca. 22.5 min, both of which were detected on all the CSF and serum electropherograms, were identified as Alb (M_r: 68 000) and a subunit of αMG (M_r: 180 000), respectively, by mixed analyses with their standard samples. Some of other peaks detected on the CSF and/or serum electropherograms were also identifiedin a similar manner (Fig. 1).

The concentration ratios of Alb and αMG in the examined CSF and serum samples were tentatively obtained by measurement of the peak area ratios on the electropherograms. The values of the αMG index in individual subjects were then calculated (see the transformed formula in parentheses in Section 1). The mean value and standard deviation (SD) of the obtained αMG index values were 0.29 and 0.16

($N=51$, range: 0.15–0.58), respectively. The distribution of the αMG index values in the various diseases and disease groups (see Section 2.1) is summarized in Table 1.

As summarized in Table 1, the mean value (±SD) of the αMG index values in patients with inflammatory disorders of the brain and/or meninges was 0.42±0.11 ($N=4$, range: 0.27–0.58), which was significantly greater ($P<0.05$) than that in those with neuropsychiatric diseases producing no organic damage in the CNS, as the control subjects (0.26±0.05, $N=15$, range: 0.17–0.36). The value in patients with cerebrovascular diseases, neurodegenerative diseases, multiple scerosis and epilepsy were not significantly different from that of the controls, although some of them, as well as three out of the four patients with the inflammatory disorders, had a value higher than the highest in the controls (Table 1). These were eight patients with acute-phased cerebral infarction (3), neurodegenerative diseases accompanied by pathological brain atrophy (1 with Altzheimer's disease, 1 with senile dementia of the Altzheimer

Table 1
Distribution of the αMG index value in various neurological disorders

Diseases or disease groups	Mean±SD	Range
Organic diseases in the CNS		
Cerebrovascular diseases (CVD)		
Cerebral infarction ($N=7$)		0.28–0.46
CVD-type dementia ($N=3$)		0.22–0.39
Total ($N=10$)	0.32±0.13	0.22–0.46
Inflammatory disorders of the brain and/or meninges		
Meningitis and encephalitis ($N=4$)	0.42±0.11	0.27–0.58
Neurodegenerative diseases		
Altzheimer's disease and senile		
Dementia of Altzheimer type ($N=4$)		0.23–0.40
Parkinson's disease ($N=4$)		0.20–0.42
Amyotrophic lateral sclerosis ($N=2$)		0.31–0.35
Others ($N=3$)		0.15–0.30
Total ($N=13$)	0.29±0.09	0.20–0.43
Multiple sclerosis ($N=5$)	0.28±0.10	0.19–0.41
Epilepsy ($N=4$)	0.29±0.07	0.22–0.38
Total ($N=36$)	0.30±0.14	0.19–0.57
Non-organic diseases in the CNS		
Peripheral neuropathy ($N=5$)	0.26 ±0.03	0.21–0.32
Psychotic disorders		
Schzophrenia ($N=3$)		0.18–0.28
Manic–depressive illness ($N=4$)		0.24–0.36
Total ($N=7$)	0.27±0.06	0.18–0.36
Neurosis and tension headache ($n=3$)	0.24±0.04	0.17–0.29
Total ($n=15$)	0.26±0.06	0.17–0.36
Total ($N=51$)	0.29±0.16	0.15–0.58

type and 1 with Parinson's disease) and 2 malignant-phased multiple sclerosis. These results suggested that BBB dysfunction, which occurs in a variety of organic diseases in the CNS, was detected by the measurement of the αMG index values obtained employing the SDS-CGE system.

Among the 51 patients treated in this study, 15 exhibited an elevated CSF TP content beyond the upper normal limit of 40 mg/dl. The highest value of 125 mg/dl was observed in the CSF of a meningitis patient having the highest αMG index value of 0.58. The αMG index value in patients with an elevated CSF TP level of 43–125 mg/dl (mean±SD: 0.34±0.14, $N=12$, range: 0.20– 0.58) was significantly greater ($P<0.05$) than that in those with a normal CSF TP content of 11–38 mg/dl (mean±SD: 0.27±0.05, $N=39$, range: 0.17–0.36). Therefore, it was suggested that the increase in the CSF proteins was caused at least partly by BBB dysfunction in association with elevation of the αMG index values. However, it has been known that the TP level in the CSF can be raised not only by the increased entry of plasma proteins due to a damaged BBB but also by the accelerated production of some proteins in the CNS [13]. Indeed, among the 12 patients with the elevated TP level, 4 with TP levels of 47–69 mg/dl exhibited low αMG values of 0.24–0.31, which were not higher than those of the controls. They were two cerebral infarction patients in the recovery phase, an multiple sclerosis patient who was approaching the malignant phase and another multiple sclerosis patient who was moving from the malignant phase to the mild phase. The increased proteins in their CSF were then surveyed by CZE, since on the SDS-CGE electropherograms of the CSF, as shown in Fig. 1A, the peaks of the IgG light chain and β-TP were not adequately separated from each other.

As shown in Fig. 2, the CZE analysis of the undenaturated CSF gave electropherograms resembling those obtained by densitometry of stained agarose-gel [14] and cellulose acetate membranes [15] except that the order of migration speed was reversed. As shown in Fig. 2A, on the electropherogram of the CSF of an multiple sclerosis patient approaching the malignant phase, the area% value of the β-globulin peak (ca. 22%) was more than two times greater than that in the CSF of the controls (7–10%) of which the typical pattern is given in Fig.

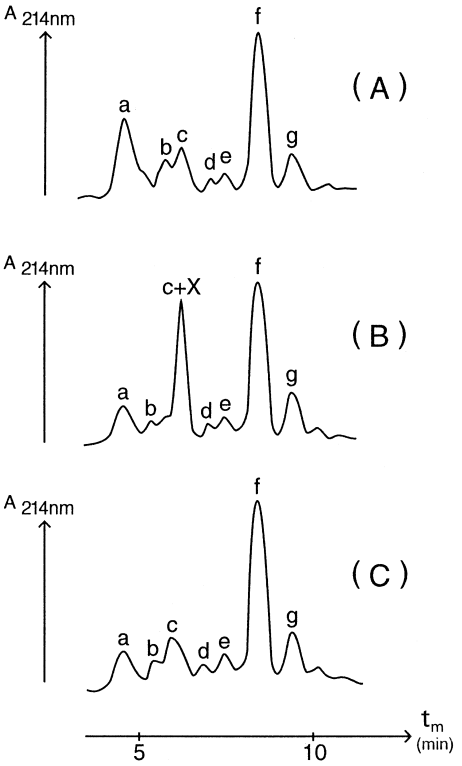

Fig. 2. CZE Electropherograms of the CSF from an multiple sclerosis patient approaching the malignant phase (A), a cerebral infarction patient in the recovery phase (B) and a neurosis patient with no organic damage in the CNS (C). Peaks: (a) γ-globulin containing IgG as the major component, (b) β_2-globulin (asialotransferrin), (c) β_1-globulin containing transferrin as the major component, (d) α_2-globulin, (e) α_1-globulin, (f) albumin, (g) prealbumin.

2C. Such a qualitative change in the proteins was not detected in the CZE analysis of the concurrent serum sample (data not shown). Therefore, it was speculated that the increase in the CSF proteins in this multiple sclerosis patient was caused mainly by the accelerated production of IgG within the CNS often observed in this disease [16]. On the other hand, as shown in Fig. 2B, in the β-globulin region of the CSF electropherograms of another multiple sclerosis patient moving from the malignant phase to the mild phase and two cerebral infarction patients in the recovery phase, a small peak, which is normally hidden in that of transferrin (Fig. 2C), was remarkably enhanced. This was identified as βTP due its disappearance by passage of the intact CSF

through a monoclonal antibody column (see Section 2.5). βTP, which has been identified as lipocalin-type prostaglandin D synthase [17,18], is synthesized in the leptomeninges and oligodendrocytes and is secreted from the choroid plexus into the CSF [19,20]. It is a sialoglycoprotein with a potent affinity to lipophilic small molecules, and works as their transporter in the CNS [21]. It has also been elucidated by our recent study employing ELISA that the βTP level in CSF is non-specifically elevated in patients who are recovering from organic damage to the CNS [22]. Therefore, increased βTP in such patients seems to have a pathophysiological role of removing waste matters and/or toxic substances formed under various pathological conditions in the CNS from the CNS to the peripheral blood flow.

These results suggested that the present SDS-CGE system is useful as an aid in the biochemical examinations of BBB function, including the elucidation as to whether elevation of the TP levels in the CSF is caused by BBB dysfunction.

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