Epilepsy & Behavior 48 (2015) 66–69

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Fibronectin is a potential cerebrospinal fluid and serum epilepsy biomarker

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ARTICLE INFO

Article history:
Received 31 October 2014
Revised 27 March 2015
Accepted 28 March 2015
Available online 8 June 2015

Keywords:
Fibronectin
Epilepsy
Cerebrospinal fluid
Serum
ELISA
Biomarker

ABSTRACT

Purpose: Previous studies have demonstrated that fibronectin (FN) levels are increased in brain tissues from patients and animals with epilepsy. This study aimed to assess FN levels in cerebrospinal fluid (CSF) and serum samples from patients with epilepsy.
Methods: Fibronectin levels were assessed in CSF and serum samples from 56 patients with epilepsy (27 and 29 individuals with intractable epilepsy and nonintractable epilepsy, respectively) and 25 healthy controls, using sandwich enzyme-linked immunosorbent assays (ELISA).
Results: CSF-FN levels were higher in patients with epilepsy (8.07 ± 1.51 mg/l versus 6.20 ± 1.18 mg/l, $p < 0.05$) than in the control group. In addition, serum-FN levels in the group with epilepsy and in the control group were 236.96 ± 65.7 mg/l and 181.43 ± 72.82 mg/l, respectively, indicating a statistically significant difference ($p = 0.01$). Interestingly, serum- and CSF-FN levels in individuals with epilepsy were not affected by antiepileptic drug and duration of epilepsy. Of note, the increase of CSF- and serum-FN levels was more pronounced in subjects with intractable epilepsy than in patients with nonintractable epilepsy.
Conclusion: Serum- and CSF-FN levels constitute a potential clinical diagnostic biomarker for epilepsy and could also be used for differential diagnosis.

1. Introduction

Fibronectin (FN) belongs to a family of high molecular weight glycoproteins that are present on the cell surface and in the extracellular fluid, connective tissue, and basement membrane [1,2]. Fibronectin participates in cell adhesion and migration by interacting with other extracellular matrix proteins and cellular ligands such as collagen, fibrin, and integrin [3].

An increase in serum-FN levels has been observed in central neuronal diseases, including multiple sclerosis [4–6], Sturge–Weber syndrome [7,8], bacterial meningitis, tick-borne encephalitis, and carcinomatous meningitis [9]. These clinical conditions can be differentiated from epilepsy. The significance of cerebrospinal fluid -fibronection (CSF-FN) in multiple sclerosis is evidenced by its presence within multiple sclerosis lesions and on macrophages in plaques [4]. Sturge–Weber syndrome can also be explained by increased expression of FN, which regulates angiogenesis and constitutes the cerebral response to chronic ischemic damage [8]. A highly significant elevation of CSF-FN levels may be an indicator of adequate host reaction and tissue repair in bacterial meningitis, tick-borne encephalitis, and carcinomatous meningitis [9]. Fibronectin also plays a critical role in neurodegenerative-related neurotrophy and neuroprotection, as well as neural differentiation and regeneration by cell matrix signal transduction [10,11]. In the above neuronal diseases, FN is considered a biochemical prognostic marker and associated with a neuroprotective role.

During a seizure, along with changes in protein synthesis in the brain, FN expression and activity were shown to increase. In addition, treatment of astrocytes with kainic acid results in decreased FN levels; in contrast, astrocyte treatment with lipopolysaccharide induces a significant increase of FN [6,12]. Considering the function of FN and its possible role in epilepsy, we hypothesized that FN may be altered in the CSF and serum concentration. Here, FN concentrations were measured in CSF and serum samples from patients with epilepsy as well as from controls who underwent lumbar punctures as part of a medical evaluation to assess the group differences.

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http://dx.doi.org/10.1016/j.yebeh.2015.03.030
1525-5050/

2. Materials and methods

2.1. Subjects

Subjects were recruited from the Epilepsy Clinic, Department of Neurology, The First Affiliated Hospital of Chongqing Medical University. Epilepsy was diagnosed and classified by two experts at our epilepsy center according to the criteria proposed by the International League Against Epilepsy in 2001. Patients underwent comprehensive clinical examination, including medical history, electroencephalography assessment, neurological and psychiatric examinations, and cranial magnetic resonance imaging (MRI). All examinations (neurological and psychiatric examinations and MRI) returned normal results. Samples from 56 patients with epilepsy (29 males and 27 females) were collected by lumbar puncture. Patient ages ranged from 13 to 70 years. Thirty-five patients had secondarily generalized tonic–clonic seizure (sGTCS), 11 had generalized tonic–clonic seizure (GTCS), 19 had complex partial seizure (CPS), and 6 had simple partial seizure (SPS) events. A total of 21, 11, and 24 patients had less than 1-year, between 1-year and 2-year, and more than 2-year history of seizure recurrence, respectively. Table 1 summarizes the seizure types and frequencies in the patients assessed here. The experimental group was subdivided into two (the group with intractable epilepsy [IE] and the group with nonintractable epilepsy [NIE]) groups. Criteria for IE included the following: seizure events for at least 2 years, use of at least three AEDs (antiepileptic drugs), and absence of structural lesion in the brain tissue as assessed by MRI and CT; patients with NIE were those with good response to AEDs and no seizure recurrence. No patient with epilepsy enrolled in this study had a seizure within 24 h of sample collection.

Control samples were obtained from 25 controls with mild dizziness and headache, no history of epilepsy or other central nervous diseases, no anxiety or depression, and not currently taking any medication. Routine clinical examination and cranial magnetic resonance imaging (MRI) did not reveal any central nervous diseases such as cerebral ischemia, hemorrhage, inflammation, and tumor. The diagnosis was carried out by two independent neurologists. Lumbar puncture was performed to further rule out central nervous system disorders. This study was approved by the Human Research Committee of Chongqing Medical University, and written informed consent forms were obtained from all participants. All procedures were conducted in accordance with the Declaration of Helsinki.

2.2. Sample collection and storage

For each subject, 2 ml of CSF and 5 ml of venous blood samples were collected. A portion of each blood sample was used by the laboratory department of our hospital for total protein and biochemical profiling. The remaining blood portion was centrifuged at 3000 *g* for 15 min for the preparation of serum, which was then aliquoted and stored at −80 °C until further analysis. Cerebrospinal fluid samples were centrifuged at 2000 *g* for 10 min at 4 °C and stored at −80 °C until use.

Table 1
Clinical characteristics of patients with epilepsy.

Characteristics	Value
Course of epilepsy (n)	
<1 year	21
1–2 years	11
>2 years	24
Seizure type (n)	
Secondarily generalized tonic–clonic seizure	35
Generalized tonic–clonic seizure	11
Complex partial seizure	19
Simple partial seizure	6
History of taking AEDs (n)	
Taking AEDs	34
Not taking AEDs	22

2.3. FN measurement

Cerebrospinal fluid- and serum-FN concentrations were determined by sandwich enzyme-linked immunosorbent assay with a rat antihuman FN ELISA kit (Abnova, USA, KA0188) according to the manufacturer's instructions and previous study [13]. Cerebrospinal fluid and serum samples were diluted 10,000 and 20,000 times, respectively. Absorbance values were determined with a Multiskan Spectrum Microplate Spectrophotometer microplate reader (Thermo Fisher Scientific, USA) at 450 nm.

2.4. Statistical analysis

Statistical analysis was performed with SPSS 19.0 (SPSS, USA). Data are mean ± standard deviation (SD). Differences between the group with epilepsy and the control group were assessed by independent samples *t*-test, while one-way analysis of variance (ANOVA) was used for additional comparisons of subtypes of patients with epilepsy (i.e., drug versus no drug; 1 versus 1–2 versus >2 years of disease duration; different seizure frequencies). $p < 0.05$ was considered statistically significant.

3. Results

Table 2 summarizes the FN concentrations obtained in CSF and serum samples from individuals with epilepsy and controls. CSF-FN concentrations in the group with epilepsy and the control group were 8.07 ± 1.51 mg/l and 6.20 ± 1.18 mg/l, respectively, indicating a statistically significant difference ($p < 0.01$). A similar trend was observed for serum samples with 236.96 ± 65.7 mg/l and 181.43 ± 72.82 mg/l FN obtained in the group with epilepsy and the control group, respectively ($p = 0.01$). Independent samples *t*-test showed that serum-FN levels were significantly higher in the group with epilepsy than in the control group ($p < 0.05$).

The differences in CSF-FN levels between the subgroup with IE (7.94 ± 1.54 mg/l) and the subgroup with NIE (8.22 ± 1.49 mg/l) were not statistically significant ($p = 0.869$). Similar results were obtained with serum-FN amounts (IE, 229.8 ± 76.18 mg/l; NIE, 244.65 ± 52.65 mg/l; $p = 0.777$). Serum- and CSF-FN levels in patients with epilepsy were not affected by AED use (Table 3). For patients with epilepsy taking AEDs, CSF-FN levels were 7.99 ± 1.43 mg/l and 8.21 ± 1.66 mg/l in those not under treatment. Serum-FN levels were 241.76 ± 56.53 mg/l and 229.54 ± 78.6 mg/l for patients using AEDs and for patients with no drugs, respectively (Table 3). Disease course seemed to have no influence on serum- and CSF-FN levels in patients with epilepsy. Serum-FN levels were 227.03 ± 79.76 mg/l, 250.60 ± 68.53 mg/l, and 239.40 ± 50.63 mg/l for patients with <1, 1–2, and >2 years of disease duration, respectively; CSF concentrations were 8.07 ± 1.75 mg/l, 7.74 ± 0.87 mg/l, and 8.27 ± 1.55 mg/l, respectively. There were no statistically significant differences among the three subgroups ($p > 0.05$) (Table 3).

Table 2
The concentration of FN in CSF and serum.

	Epilepsy (n = 56)	Control (n = 25)	p-Value*
CSF-FN (mg/l)	8.07 ± 1.51	6.20 ± 1.18	<0.01
Serum-FN (mg/l)	236.96 ± 65.7	181.43 ± 72.82	=0.01