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Variations in the cerebrospinal fluid proteome following traumatic brain injury and subarachnoid hemorrhage

David E. Connor Jr. (DO)[a], Ganta V. Chaitanya (PhD)[b], Prashant Chittiboina (MD, MPH)[c], Paul McCarthy (MD, FCCP)[d], L. Keith Scott (MD, MSc)[e], Lisa Schrott (PhD)[f], Alireza Minagar (MD)[g], Anil Nanda (MD, MPH)[h], J. Steven Alexander (PhD)[i,*]

[a] Baptist Health Neurosurgery Arkansas, Little Rock, AR, United States
[b] Cardiovascular Research Center, University of Virginia, Charlottesville, VA, United States
[c] Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke, Bethesda, MD, United States
[d] Department of Medicine, Sect. of Nephrology, University of Maryland, Baltimore, MD, United States
[e] Department of Critical Care Medicine, Louisiana State University Health Sciences Center-Shreveport, LA, United States
[f] Department of Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center-Shreveport, LA, United States
[g] Department of Neurology, Louisiana State University Health Sciences Center-Shreveport, LA, United States
[h] Department of Neurosurgery, Louisiana State University Health Sciences Center-Shreveport, LA, United States
[i] Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center-Shreveport, LA, United States

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ABSTRACT

Background: Proteomic analysis of cerebrospinal fluid (CSF) has shown great promise in identifying potential markers of injury in neurodegenerative diseases [1–13]. Here we compared CSF proteomes in healthy individuals, with patients diagnosed with traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH) in order to characterize molecular biomarkers which might identify these different clinical states and describe different molecular mechanisms active in each disease state.
Methods: Patients presenting to the Neurosurgery service at the Louisiana State University Hospital-Shreveport with an admitting diagnosis of TBI or SAH were prospectively enrolled. Patients undergoing CSF sampling for diagnostic procedures were also enrolled as controls. CSF aliquots were subjected to 2-dimensional gel electrophoresis (2D GE) and spot percentage densities analyzed. Increased or decreased spot expression (compared to controls) was defined in terms of in spot percentages, with spots showing consistent expression change across TBI or SAH specimens being followed up by Matrix-Assisted Laser Desorption/Ionization mass spectrometry (MALDI-MS). Polypeptide masses generated were matched to known standards using a search of the NCBI and/or GenPept databases for protein matches. Eight hundred fifteen separately identifiable polypeptide migration spots were identified on 2D GE gels. MALDI-MS successfully identified 13 of 22 selected 2D GE spots as recognizable polypeptides.
Results: Statistically significant changes were noted in the expression of fibrinogen, carbonic anhydrase-I (CA-I), peroxiredoxin-2 (Prx-2), both α and β chains of hemoglobin, serotransferrin (Tf) and N-terminal haptoglobin (Hp) in TBI and SAH specimens, as compared to controls. The greatest mean fold change among all specimens was seen in CA-I and Hp at 30.7 and −25.7, respectively. TBI specimens trended toward greater mean increases in CA-I and Prx-2 and greater mean decreases in Hp and Tf.
Conclusions: Consistent CSF elevation of CA-I and Prx-2 with concurrent depletion of Hp and Tf may represent a useful combination of biomarkers for the prediction of severity and prognosis following brain injury.

1. Background

More than 1.5 million American civilians suffer traumatic brain injury (TBI) annually with immediate and long-term sequel ranging from chronic headaches to significant disability and, for some, ultimately death in as many as 50,000 cases per year [14,15]. While aneurysmal subarachnoid hemorrhage (SAH) is significantly less

* Corresponding author.
E-mail addresses: dconnor1979@gmail.com (D.E. Connor Jr), vg6u@virginia.edu (G.V. Chaitanya), prashant.chittiboina@nih.gov (P. Chittiboina), pbluesmd@yahoo.com (P. McCarthy), lscott2@lsuhsc.edu (L.K. Scott), lisaschrottl@gmail.com (L. Schrott), aminag@lsuhsc.edu (A. Minagar), ananda@lsuhsc.edu (A. Nanda), jalexa@lsuhsc.edu (J.S. Alexander).

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frequent, at 18–25,000 cases (6–8 cases/100,000) annually, the long term outcomes can be equally as grave [16–18]. Multiple radiographic and clinical indices have been proposed over the years in an attempt to predict the severity of brain injury and provide an ultimate prognosis for meaningful recovery with varying levels of success [19–23].

Proteomic analysis offers a new and powerful technique for the systematic and specific identification of target proteins within a given sample. We have previously reported changes in the endothelial proteome in multiple sclerosis (MS) and in response to NMDA stimulation [24,25]. Identification of CSF proteomic targets that are altered during stress and disease is an important goal for designating their etiologic basis and diagnostic utility [26]. Analysis of cerebrospinal fluid (CSF) has been used to identify potential markers of injury and study pathophysiologic changes in neurologic diseases ranging from MS to Alzheimer's disease [1–13]. This technique offers the potential not only to identify biomarkers of the severity of brain tissue injury in TBI and SAH, but also may ultimately lead to the development of mechanism-based antagonists that can be used to ameliorate the degree of secondary injury common to the pathophysiology of both disease states.

2. Methods

2.1. Study approval

Prior to the collection of data, our protocol was reviewed and approved on June 2, 2009 by the Institutional Review Board for the Protection of Human Research Subjects at the Louisiana State University Health Sciences Center-Shreveport under Protocol # H09-138.

2.2. Sample acquisition

Following informed consent, consecutive patients presenting to the neurosurgery service at the Louisiana State University Hospital-Shreveport with the admitting diagnosis of either traumatic brain injury (TBI) or subarachnoid hemorrhage (SAH) were enrolled in the following investigation. Additional patients undergoing cerebrospinal fluid sampling for diagnostic procedures including lumbar puncture or myelography were enrolled as controls.

All cases of TBI involved clinical history consistent with closed head injury secondary to motor vehicle collision, fall, or blunt trauma. Non-contrast computed tomography (CT) scan of the head confirmed a pattern of cerebral edema with or without intraparenchymal or subarachnoid hemorrhage. Patients with extra-axial, compressive hematomas were excluded. All cases of SAH were identified based upon a combination of clinical history of sudden headache, new onset seizure, or focal neurologic deficit, as well as a non-contrast CT scan of the head with evidence of cisternal or parenchymal hemorrhage consistent with ruptured cerebral aneurysm.

All patients underwent cerebrospinal fluid (CSF) diversion via external ventricular catheter (EVD) during the normal course of their clinical management. In cases of TBI, EVD placement was performed for the monitoring and treatment of elevated intracranial pressure; SAH patients underwent drainage for the management of early hydrocephalus or the presence of significant intraventricular hemorrhage.

Specimens were collected between 4 and 18 h following EVD placement by aspirating CSF from the reservoir drainage bag. Two additional control samples were selected from discarded specimens from patients undergoing CSF analysis or access for diagnostic procedures including lumbar puncture or myelography. These specimens were screened with cell counts, as well as for glucose and protein levels, and were excluded if they showed evidence of infection or neoplastic process. Samples were also excluded in cases with clinical history of rheumatoid arthritis, metastatic neoplasm, or diabetes mellitus. All CSF aliquots were centrifuged to remove formed blood elements and the supernatants were subsequently frozen and stored at −80 °C pending analysis.

2.3. Total protein determination

Total protein was calculated for all experimental and control specimens using the DC Protein Assay (Bio-Rad Laboratories, Hercules, CA) as previously described [27,28]. Protein concentration standards were prepared using six serial dilutions of 2 mg/mL bovine serum albumin. Five μL CSF samples were mixed in a standard 96-well microplate with 25 μL of reagent A (1–5% NaOH) and 200 μL of Reagent B (Folin reagent) and mixed for 15 min on a plate agitator. Next, the microplate was read at 690 nm using a Finstruments Multiskan model MCC340 (MTX Lab Instruments, Vienna, VA) microplate reader. A standard curve was prepared using absorbance data from BSA dilutions, from which a 3rd-order polynomial equation was identified. This was then used to calculate experimental protein concentrations.

2.4. Sample preparation

All specimens underwent albumin and immunoglobulin depletion using the Calbiochem ProteoExtract Albumin/IgG Removal kit (Cat. # 122642). Remaining samples were then analyzed using the BCA assay (Pierce Chemical, Rockford, IL) to determine total protein concentration as previously reported [29]. All samples were subsequently subjected to TCA precipitation, with product dissolved to 1 mg/mL in 1:1 diluted SDS boiling buffer:urea sample buffer.

2.5. Two-dimensional electrophoresis

Two-dimensional gel electrophoresis (2D GE) was performed using the carrier ampholine method of isoelectric focusing [30,31] developed by Kendrick Labs, Inc. (Madison, WI). Isoelectric focusing was carried out in a glass tube of inner diameter 3.3 mm using 2% pH 4–8 mix Servalytes (Serva, Heidelberg Germany) for 20,000 V h. An aliquot of 100 ng of an IEF internal standard, tropomyosin, was added to the sample. This protein migrates as a doublet with a lower polypeptide spot of MW 33,000 and p*I* 5.2; an arrow on the stained gel marks its position. The enclosed tube gel pH gradient plot for this set of ampholines was determined with a surface pH electrode.

After equilibration for 10 min in Buffer'O' (10% glycerol, 50 mM dithiothreitol, 2.3% SDS and 0.0625 M Tris, pH 6.8), each tube gel was sealed to the top of a stacking gel that overlaid a 10% acrylamide slab gel (1.00 mm thick). SDS slab gel electrophoresis was carried out for about 5 h at 25 mA/gel. The following proteins (Sigma Chemical Co., St. Louis, MO) were used as molecular weight (MW) standards: myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000). These standards appear along the basic edge of the silver-stained [32] 10% acrylamide slab gel. The silver stained gels were dried between sheets of cellophane with the acid edge to the left.

2.6. Computerized comparisons

Duplicate gels were obtained from each sample and were scanned with a laser densitometer (Model PDSI, Molecular Dynamics Inc., Sunnyvale, CA). The scanner was checked for linearity prior to scanning with a calibrated Neutral Density Filter Set (Melles Griot, Irvine, CA). The images were analyzed using Progenesis Same