European Psychiatry 29 (2014) 307–315

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Plasma-based proteomics reveals lipid metabolic and immunoregulatory dysregulation in post-stroke depression

Y. Zhan [b,c,1], Y.-T. Yang [a,b,c,1], H.-M. You [b,c,1], D. Cao [a,b,c,1], C.-Y. Liu [b,c], C.-J. Zhou [a,b,c], Z.-Y. Wang [b,c], S.-J. Bai [b,c], J. Mu [a,b,c], B. Wu [b,c], Q.-L. Zhan [d], P. Xie [a,*,b,c]

[a] *Department of Neurology, The First Affiliated Hospital at Chongqing Medical University, 1, Yixue Road, Yuzhong District, Chongqing, 400016, China*
[b] *Chongqing Key Laboratory of Neurobiology, Chongqing, China*
[c] *Institute of Neuroscience, Chongqing Medical University, Chongqing, China*
[d] *Department of Neurology, The Fifth People's Hospital of Chongqing, Chongqing, China*

ARTICLE INFO

Article history:
Received 5 October 2013
Received in revised form 23 March 2014
Accepted 26 March 2014
Available online 19 May 2014

Keywords:
Post-stroke depression
PSD
Stroke
Plasma
Proteomic

ABSTRACT

Background: Post-stroke depression (PSD) is the most common psychiatric complication facing stroke survivors and has been associated with increased distress, physical disability, poor rehabilitation, and suicidal ideation. However, the pathophysiological mechanisms underlying PSD remain unknown, and no objective laboratory-based test is available to aid PSD diagnosis or monitor progression.
Methods: Here, an isobaric tags for relative and absolute quantitation (iTRAQ)-based quantitative proteomic approach was performed to identify differentially expressed proteins in plasma samples obtained from PSD, stroke, and healthy control subjects.
Results: The significantly differentiated proteins were primarily involved in lipid metabolism and immunoregulation. Six proteins associated with these processes – apolipoprotein A-IV (ApoA-IV), apolipoprotein C-II (ApoC-II), C-reactive protein (CRP), gelsolin, haptoglobin, and leucine-rich alpha-2-glycoprotein (LRG) – were selected for Western blotting validation. ApoA-IV expression was significantly upregulated in PSD as compared to stroke subjects. ApoC-II, LRG, and CRP expression were significantly downregulated in both PSD and HC subjects relative to stroke subjects. Gelsolin and haptoglobin expression were significantly dysregulated across all three groups with the following expression profiles: gelsolin, healthy control > PSD > stroke subjects; haptoglobin, stroke > PSD > healthy control.
Conclusions: Early perturbation of lipid metabolism and immunoregulation may be involved in the pathophysiology of PSD. The combination of increased gelsolin levels accompanied by decreased haptoglobin levels shows promise as a plasma-based diagnostic biomarker panel for detecting increased PSD risk in post-stroke patients.

1. Introduction

Post-stroke depression (PSD) is the most common psychiatric complication facing stroke survivors and is estimated to affect at least 30% of the post-stroke population [1,45,73]. PSD has deleterious effects on motivation and cognitive function [60] and has been shown to have a negative impact on functional recovery [57]. Currently, PSD diagnosis solely relies on subjective identification of clinical symptoms according to DSM-IV criteria (American Psychiatric Association 2001) [5]. PSD patients are often underdiagnosed due to the physical and cognitive impairments that can hinder performance during psychiatric interviews [65]. To address this issue, an objective laboratory-based test for PSD would be useful in improving diagnosis rates. To this end, a better understanding of the pathophysiology underlying PSD is essential to improving diagnostic tools and pharmacotherapies for this disorder.

Proteomics – the analysis of protein expression in biosamples – can improve our understanding of pathophysiological mechanisms and aid in diagnostic tool development [29]. The peripheral bloodstream, as a "sentinel tissue" [42], is linked to other proteomes that continually diffuse into the circulation. Moreover, deranged blood-brain barrier (BBB) permeability has been reported in major depressive disorder (MDD) [26], implying protein exchange between the brain and the peripheral circulation.

* Corresponding author. Tel.: +86 23 68485490; fax: +86 23 68485111.
E-mail address: xiepeng@cqmu.edu.cn (P. Xie).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.eurpsy.2014.03.004
0924-9338/

Therefore, the plasma proteome may hold pathophysiological information associated with PSD. As previous studies have demonstrated that both stroke [20,79] and depression [11,75] display plasma protein abnormalities, we hypothesize that a proteomic signature for PSD can be detected in human plasma.

In this study, plasma samples from PSD, stroke, and healthy control (HC) subjects were analyzed using a quantitative proteomic approach based on isobaric tags for relative and absolute quantitation (iTRAQ) and multi-dimensional liquid chromatography-tandem mass spectrometry (LC-MS/MS). Differentially expressed proteins were further validated by Western blotting and analyzed by Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources. This proteomic approach may ultimately contribute to a better understanding of the pathophysiology underlying PSD.

2. Subjects and method

2.1. Subjects and ethics statement

First, 35 PSD and 35 stroke subjects were enrolled at one month post-stroke in the Department of Neurology of the First Affiliated Hospital at Chongqing Medical University (Chongqing, China) between July 2011 and June 2012. Candidates with both ischemic and hemorrhagic stroke were recruited. Candidates who were not capable of completing the psychiatric interview or scale examination were excluded. The clinical diagnosis of stroke was performed by a neurologist in accordance with World Health Organization (WHO) criteria [69] and confirmed by computerized tomography (CT) or magnetic resonance imaging (MRI). The average time from stroke onset to admission was 10.22 ± 6.54 hours (1–24 hours). Exclusion criteria for PSD and stroke subjects included patients with:

- a known history of depression prior to stroke, current antidepressant treatment, alcohol abuse, or any concomitant psychiatric illness;
- a serious pathogenic condition and/or with dementia, aphasia, dysarthria, and deafness severe enough that they were unable to participate in clinical psychological testing;
- Parkinson's disease;
- infectious or inflammatory diseases;
- severe concomitant physical disease;
- hepatic, renal, hematological, immunological, or thyroid disorders and/or severe complications thereof.

In addition, 35 HC subjects were recruited in the medical examination center of the First Affiliated Hospital at Chongqing Medical University. HC were required to have no current or previous lifetime history of neurological, DSM-IV Axis I/II, and/or systemic medical illness. Written informed consents were obtained from all participants. The Ethical Committee of Chongqing Medical University reviewed and approved the protocol of this study and the procedures employed for sample collection.

Severity of stroke was assessed by trained neurologists using NIHSS at admission, before discharge [9]. Functional outcomes were evaluated with the modified Rankin Scale (mRS). In agreement with the literature, a favorable outcome was defined as a mRS score of less than or equal to three and BI G75 [72]. Cognition (Mini-Mental State Examination [MMSE]) was assessed once within the first three days after admission.

Psychological evaluation was performed by a single trained psychologist at approximately seven days and one month after stroke onset. Depression was diagnosed according to the DSM-IV criteria, and the severity of depressive symptoms were assessed by a Hamilton Depression Rating Scale (HDRS), score of greater than 8 [17].

2.2. Sample collection

Blood samples (5 ml) were collected in EDTA-vacutainers (BD vacutainers catalog #367863) by venipuncture between 8:00–10:00 A.M., immediately placed on ice, and centrifuged at 3000 rpm for 15 min at 4 °C. The resultant plasma was aliquoted and stored at −80 °C within one hour of collection.

2.3. Protein digestion and iTRAQ labeling

Pooled plasma samples were generated by combining equal volumes of the 15 individual plasma samples from every group ($n = 15$). According to the manufacturer's instructions, 450 μl from each pool was depleted of the most abundant proteins with a MARS-human 14 HPLC column (Agilent, Santa Clara, CA, USA) for iTRAQ. The proteins from the immunodepletion column flow-through were ultrafiltered (5 kD). Then, 120 μg of proteins from each sample were incorporated into 30 μl STD buffer (4% SDS, 100 mM DTT, 150 mM TrisHCl pH 8.0), incubated in boiling water for 5 min, cooled to room temperature, diluted with 200 μl UA buffer (8 M urea, 150 mM TrisHCl pH 8.0), and transferred to 30 kD ultrafiltration. The samples were centrifuged at 14,000 g for 15 min, and 200 μl UA buffer was added. The samples were centrifuged for 15 min at the same conditions. Then, 100 μl 0.05 M iodoacetamide in UA buffer was added, and the samples were incubated for 20 min in the dark. After 10 min centrifugation at the above conditions, the filters were washed three times with 100 μl UA buffer. Then, 100 μl DS buffer (50 mM triethylammoniumbicarbonate at pH 8.5) was added to the filters, and the samples were centrifuged for 10 min at the same conditions. This step was repeated twice. Finally, 2 μg trypsin (Promega) in 40 μl DS buffer was added to each filter. The samples were incubated overnight at 37 °C. The resulting peptides were collected by centrifugation. The filters were rinsed with 40 μl 10 × DS buffer again. iTRAQ labeling was performed according to the manufacturer's instructions (Applied Biosystems). iTRAQ reagents 115 and 118 were used for PSD subjects, 116 and 119 for stroke subjects, and 117 and 121 for HC subjects. Each respective sample consisted of a pool of plasma from 15 PSD, 15 stroke, or 15 HC subjects. The analytic processes were repeated twice, including plasma depletion, protein digestion, iTRAQ labeling, SCX fractionation, and LC-MS/MS analysis.

2.4. Peptide fractionation with strong cation exchange chromatography

Prior to LC-MS/MS analysis, peptides were purified from excess labeling reagent by strong cation exchange (SCX) chromatography: peptides were dried in a vacuum concentrator, dissolved in strong cation exchange buffer A (10 mM KH_2PO_4 in 25% of ACN, pH 3.0), and loaded onto a Polysulfoethyl 4.6 × 100 mm column (5 μm, 200 Å, Poly LC Inc., Columbia, MD, USA) at a flow rate of 1 ml/min. A suitable gradient elution was applied to separate peptides at a flow rate of 1 ml/min with Elution buffer (10 mM KH_2PO_4, 500 mM KCl in 25% acetonitrile, pH 3.0). Eluted peptides were collected and desalted by an offline fraction collector. The resulting fractions were combined into ten pools and desalted on C18 Cartridges (Empore™ SPE Cartridges C18 (standard density), bed I.D. 7 mm, volume 3 ml, Sigma, St. Louis, MO, USA). Each final fraction was concentrated by a vacuum concentrator and resuspended in 40 μl of 0.1% (v/v) trifluoroacetic acid. All samples were stored at −80 °C until LC-MS/MS analysis.