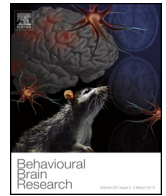




Contents lists available at ScienceDirect

Behavioural Brain Research

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



Research report

Protein profiling in serum after traumatic brain injury in rats reveals potential injury markers

Eric Peter Thelin (MD, PhD)^{a,*}, David Just^{b,1}, Arvid Frostell (MD)^a,
Anna Häggmark-Månberg (PhD)^b, Mårten Risling (MD, PhD)^c,
Mikael Svensson (MD, PhD)^{a,d}, Peter Nilsson (PhD)^b, Bo-Michael Bellander (MD, PhD)^{a,d}

^a Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

^b Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH-Royal Institute of Technology, Stockholm, Sweden

^c Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

^d Department of Neurosurgery, Karolinska University Hospital, Stockholm, Sweden

HIGHLIGHTS

- Profiling of serum proteome could reveal potential brain injury markers.
- The serum proteome present different characteristics over time after injury.
- Complement proteins are increased in serum early after injury.
- Clusters of proteins were identified, indicating co-variance.
- Hypoxic brain injury led to a general upregulation of proteins in serum.

ARTICLE INFO

Article history:

Received 31 May 2016

Received in revised form 21 July 2016

Accepted 29 August 2016

Available online xxx

Keywords:

Traumatic brain injury

Hypoxia

Serum proteins

Biomarkers

Protein-array

ABSTRACT

Introduction: The serum proteome following traumatic brain injury (TBI) could provide information for outcome prediction and injury monitoring. The aim with this affinity proteomic study was to identify serum proteins over time and between normoxic and hypoxic conditions in focal TBI.

Material and methods: Sprague Dawley rats (n=73) received a 3 mm deep controlled cortical impact ("severe injury"). Following injury, the rats inhaled either a normoxic (22% O₂) or hypoxic (11% O₂) air mixture for 30 min before resuscitation. The rats were sacrificed at day 1, 3, 7, 14 and 28 after trauma. A total of 204 antibodies targeting 143 unique proteins of interest in TBI research, were selected. The sample proteome was analyzed in a suspension bead array set-up. Comparative statistics and factor analysis were used to detect differences as well as variance in the data.

Results: We found that complement factor 9 (C9), complement factor B (CFB) and aldolase c (ALDOC) were detected at higher levels the first days after trauma. In contrast, hypoxia inducing factor (HIF)1 α , amyloid precursor protein (APP) and WBSR17 increased over the subsequent weeks. S100A9 levels were higher in hypoxic-compared to normoxic rats, together with a majority of the analyzed proteins, albeit few reached statistical significance. The principal component analysis revealed a variance in the data, highlighting clusters of proteins.

Conclusions: Protein profiling of serum following TBI using an antibody based microarray revealed temporal changes of several proteins over an extended period of up to four weeks. Further studies are warranted to confirm our findings.

© 2016 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Background

Traumatic brain injury (TBI) is globally a common cause of death especially among the young but due to socio-demographic changes it is also increasing among the frail elderly [1]. The injury progression does not stop at impact [2] but progress over time and harmful secondary events may further deteriorate patients affected [3,4]. One of these secondary insults is hypoxia at the scene of accident,

* Corresponding author.

E-mail addresses: eric.thelin@ki.se (E.P. Thelin), david.just@scilifelab.se (D. Just), arvid.frostell@ki.se (A. Frostell), anna.haggmark@scilifelab.se (A. Häggmark-Månberg), marten.risling@ki.se (M. Risling), mikael.svensson@ki.se (M. Svensson), peter.nilsson@scilifelab.se (P. Nilsson), bo-michael.bellander@ki.se (B.-M. Bellander).

¹ Authors contributed equally.

<http://dx.doi.org/10.1016/j.bbr.2016.08.058>

0166-4328/© 2016 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

which may be caused by TBI, obstructive airways or other thoracic trauma, and is present in up to 35% in human TBI patients [5] and has been shown to result in an altered neuroinflammatory response and an unfavorable outcome [6,7].

In order to improve diagnostics and management of patients suffering from TBI, protein biomarkers that monitor cerebral pathophysiology have been introduced [8,9]. While the cerebrospinal fluid (CSF) proteome is in close proximity to the central nervous system [10], it is difficult to acquire. Moreover, with the discovery of the glymphatic system and the flow of proteins from the central nervous system to blood [11], it has become more interesting to assess the post-traumatic proteomic spectrum in serum [12].

The most studied serum protein biomarker is the primarily astrocytic, calcium-binding protein S100B where levels have been shown to correlate to patient outcome [13], been useful for monitoring the potential development of secondary injuries [14] and screening for potentially harmful intracranial injury in the emergency room [15]. However S100B is also expressed in other tissues such as fat, skin and bone [16] confounding interpretations of serum levels in patients with more extensive extracranial trauma [17]. Therefore, more brain specific proteins in serum have been suggested to better assess cerebral injury, including Myelin Basic Protein (MBP) [18], Glial-Fibrillary Acidic Protein (GFAP) [19] and Neuron Specific Enolase (NSE) [20]. Moreover, combinations of proteins may further improve outcome prediction [21] as well as provide a better injury stratification [22].

While analysis of brain specific proteins currently are being implemented into the clinic to evaluate cerebral injury, the research focus is shifting to the assessment of the underlying pathophysiology in TBI. The inflammatory response has been shown to play an important role [23,24] with involvement of pro-inflammatory components of the innate immune system, including complement factors and cytokines [25–30] especially in relation to hypoxic conditions [31]. Studies of serum mediators of inflammation in TBI over a prolonged period of time are limited, since samples commonly are collected only the first hours and day following trauma [32,33] perhaps missing the complete inflammatory process.

The complex pathophysiological mechanisms that transpire during the different phases of TBI are complicated to monitor in the affected patient. To improve assessment, proteomic analyses of selected proteins have been suggested to provide novel insight into disease pathology [34–37]. Mass-spectrometry approaches as well as protein arrays for monitoring of different pathophysiological processes in serum following TBI have been used in both pre-clinical and clinical studies [38–40]. However, many of these have been limited in sample size, and in the case of antibody arrays limited in the number of analyzed proteins.

In our study, we have utilized the extensive resource of antibodies generated within the Human Protein Atlas [41] for analysis of serum proteins in a model of hypoxic TBI in rats up to 28 days following TBI. The primary aim of this study was to compare rats with and without a hypoxic insult. As a secondary aim, the proteins were analyzed by oxygenation state, temporal profile after trauma and how they differed between naïve, sham and TBI animals.

2. Material and methods

2.1. Study sample

The included animals were female Sprague-Dawley (SD) rats, about 15 weeks old weighing 251 g in average. The serum was acquired from rats where the experimental procedure was performed in the scope of a previous study by our group [42]. The rats were kept in 12/12 h dark/light cycle, normal temperature (21 °C) and air humidity. Food and water were provided ad libitum. The

Swedish Department of Agriculture approved the ethical permission to perform the study (#N369/12 and #126/13).

2.2. Traumatic brain injury model

As previously and thoroughly described, we used a controlled cortical impact (CCI) model that hit exposed dura of a sedated and mechanically ventilated animal [42]. The piston hit the animal in the right parieto-frontal area with a depth of 3 mm, constituting to what is considered a “severe” focal traumatic brain injury [43,44]. Following impact, the animal was exposed to either normoxic (22% O₂) or hypoxic (11% O₂) gas mixtures for 30 min as the wound was sutured. The isoflurane was then discontinued, the animal awoken and returned to the home cage after having fully regained consciousness. The injury model, and resulting lesion, is thoroughly described in our previous publication, including a movie of the surgical procedure [42]. In the original experiment, five rats were used per oxygenation group (hypoxia/normoxia) and were sacrificed at day 1, 3, 7, 14 and 28 (n = 50 in total). Moreover, five day 1 and five day 7 normoxia and hypoxia shams were also used (n = 20 in total), as well as three naïve animals (a total of n = 73 rats). The naïve animals had the same sex, age (15 weeks) and weight (average: 248 g) as the experimental and sham animals.

2.3. Serum extraction

Shortly following euthanasia, the left ventricle of the heart was punctured using an angiocatheter (18G, KD Medical, Berlin, Germany). Sampling tubes were placed vertically for 60 min to allow separation of coagulation components. After that, the samples were centrifuged (Spectrafuge 16M[®], Labnet International, Edison, USA) for 10 min at 10.000g and the serum stored in –80°C until analysis. Unfortunately, five rats (n = 2 day 7 normoxia, n = 2 day 7 hypoxia and n = 1 day 14 normoxia) did not provide adequate sample volumes and were therefore excluded from the study.

2.4. Creation of a targeted antibody suspension bead array

Proteins to be analyzed were selected based on potential association to TBI according to previously published studies (see Supplementary information 1). The proteins represented a variety of functions, including structural damage to cerebral tissue, the inflammatory response, metabolic cascades, cellular signaling, angiogenesis as well as several S100 proteins involved in both cerebral and non-cerebral functions. In total 204 antibodies targeting 143 unique proteins, were selected from the Human Protein Atlas (www.proteinatlas.org) (Fig. 1). In silico designed protein fragments (PrESTs) of roughly 80–100 amino acids are produced in E.Coli and purified before immunized into rabbits for antibody production. Subsequently, rabbit sera are purified on the respective antigen for antibody purification [45,46]. Immobilization of antibodies to beads was performed as previously described [47] by activating the magnetic color-coded beads (500 000 beads per identity, MagPlex Luminex Corp.) by using 0.1 M sodium hydrogen phosphate, 0.5 mg of sulfo-NHS (Nordic Biolabs) and 0.5 mg EDC (ProteoChem) per antibody. The activated beads were then incubated for 20 min on a shaker and subsequently washed with MES-buffer. A total concentration of antibody added to the beads was 17.5 ng/μl, with each antibody being assigned a specific bead ID. The coupling was allowed to occur for 2 h at ambient temperature. After washing off unbound protein fragments using 0.1% Tween-20, the beads were blocked using Roche blocking reagent for ELISA (supplemented with 400 μl Tween20) overnight and the different bead identities were combined into a suspension bead array.

متن کامل مقاله

دریافت فوری ←

ISIArticles

مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات