Diagnostics of Brain Rehabilitation

Nathalie Spita
Virginia Commonwealth University

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Abstract

Traumatic brain injury (TBI) is a leading cause of long-term morbidity among the young resulting in significant societal impacts. Yet advances in TBI therapeutic care have been largely limited by the complexity of the pathobiology, heterogeneity among patients, and imprecise endpoint assessments with which to evaluate efficacy. Thus, there remains a significant need for improved diagnostics, particularly for guiding novel therapeutic use and outcomes. So-called therapeutic assays are of particular interest in the new area of TBI rehabilitation, which ideally would target a window of heightened brain plasticity during which circuit remodeling would support recapitalization of lost function. The biochemical processes associated with brain plasticity following TBI produce metabolized components that are small enough in size to passively diffuse into peripheral fluid and by natural means are excreted into urine. We employ high performance mass spectrometry to quantify these byproducts, comprising a "TBI urinary signature" of some 2,500 TBI selective molecules. In this study we hypothesized that the urinary signature would evolve with the advent of a plasticity window during the course of inpatient rehabilitation. Urine specimens were placed at 4°C after collection and centrifuged at 1500g and 4°C for 15 min. Aliquots were then stored at -80°C.

Study Design & Sample Collection

A controlled demographic of young adult Caucasian male subjects was recruited with informed consent and approval by the Virginia Commonwealth University Institutional Review Board (Richmond, VA). TBI subjects were enrolled upon admission to inpatient rehabilitation at a mean 22 days post injury (n=8; 26±6 years old). Criteria excluded subjects with non-cranial bone fractures, renal dysfunction at time of rehabilitation admission, and a positive history for past brain injury or neurological disease. Admission to the Brain Injury Rehabilitation Unit was based on standards of care for demonstrating readiness, with required medical stability and capacity to progress in an acute rehabilitation program. Beginning at the 72 hours on unit, three mid-stream urine specimens were acquired within a 48-h window. Subsequently an additional three mid-stream urine specimens were collected within a 48-h window approaching discharge of the subject from the Brain Injury Rehabilitation Unit. Urine specimens were passed at 4°C after collection and centrifuged at 1500g and 4°C for 15 min. Aliquots were then stored at -80°C.

Sample Processing

Specimens (six per TBI subject and three per control) were load-normalized to an osmolarity measure of 130 mOsm/kg with Nanopure water. Balanced speciments (100 µL) were filtered with 0.1 µm pore Ultrafilters-MC units (Millipore, Billerica, MA), with the supernatants transferred to vials for direct injection (8 µL on column) in a group-interpersed order. Reverse-phase separation was performed with a nano-Acquity chromatography system, using a Symmetry C18 trapping column (2 cm x 190 µm i.d.) and an HSS T3 nanoColumn (15 cm x 75 µm i.d.) capillary column (Waters, Milford, MA). Components were gradient separated using 0.1% formic acid modified acetonitrile and water. Eluting analytes were electrosprayed into a Synapt G2 hybrid ion mobility/mass spectrometer (Waters), operated in a data-independent analysis mode. All analytical work was performed within a climate-controlled clean room.

Design and Methods

Data processing were presented using PLGS software v2.5.2 (Waters). Accurate mass and retention time (AMRT) tables for triplicate specimens were merged to generate a single composite molecular profile per subject that accounted for intraday variance. All subject profiles were aligned by AMRT values (±2 ppm mass accuracy; ±0.5 min retention time) using Expressions software v2.5.2. Non-reproducing AMRT measures (<2%) were removed. Values from a simulated Gaussian distribution were randomized about the limit of quantification were imputed to left-censored data denoting a non-random group-specific level below the detection limit. Inter-subject normalization (median intensity, 1000 most intense ions) and log2 transformation procedures were performed.

Statistical Analysis

Aligned composite molecular profiles (separate admission and discharge profiles per TBI subject) were statistically tested using the MultiExperimentViewer (v. 4.8.1) informatics package for array data. Volcano plot presentations were generated following a Welch's t-test method with alpha adjusted to 0.01. The TBI-responsive "Uromic" was evaluated across all specimens using a non-parametric Kruskal-Wallis method with alpha adjusted to 0.01, with results presented in a heatmap format. Principle component analysis was performed for data-reduction, with the first two components plotted.

Results

Heatmap of differential molecular factors within the TBI urinary signature ("Uromic"). Top: heatmap comprised of molecular factors selectively present during admission to brain injury rehabilitation. Bottom: heatmap comprised of molecular factors selectively present during discharge from brain injury rehabilitation. These results are illustrated in the "regeneration axis" in Figure 2, classifying specimens based on time post-TBI / within rehabilitation. Red = increased, green = decreased, relative to non-traumatized control subjects. Kruskal-Wallis p<0.01.

Conclusions

The TBI urinary signature is distinct from matched controls. Further, the TBI urinary signature evolves with time in recovery. Work is ongoing to evaluate the clinical correlation of the temporally distinct signatures and the precise nature of the underlying constituents. To date we have determined that the TBI urinary signature includes a compilation of peptides. These peptides are by-products from proteins with an enriched relevance to neuroplasticity as illustrated in the table below. Many peptides are derivatives from the terminal signaling motif of the parent protein, denoting functional significance to their release during a timeframe critical to rehabilitative intervention and rewiring of circuits associated with lost function.

Abstract

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Nathalie A. Spita, Jillian E. Staffberg and Andrew K. Otten

Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA 23298

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