Biomarkers of sub-acute and chronic phases following traumatic brain injury

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Abstract

Each year more than 2 million Americans sustained a traumatic brain injury (TBI) and estimated 5.3 million currently live with disabilities from brain injury. There exists an urgent need to develop and refine biological markers of acute injury and chronic recovery after TBI. In this study, we focused primarily on the dynamic molecular events underlying the sub-acute (7 to 14 days) and chronic (14 to 30 days) phases after brain injury. Four categories of mechanism-based potential biomarkers, which include delayed cell death and autophagy, inflammation biomarkers, axonal injury and regeneration and stem/progenitor marker have been investigated on the brain samples within thirty days post-TBI in a rat controlled cortical injury model.

Our results show that there are several viable biomarker candidates that can be post acute (up to 7 days) injury. For example, the markers for activated astrocytes (GFAP) were found to accumulate after 7 days and persisted for 30 days post-TBI. Interestingly, the level of the CNPase (marker of oligodendrocytes) also increased after 7 days, but returned to control levels by 14 days. Interesting, the marker of myelin (MBP) was dramatically decreased after 1day post-TBI. However, the level of MBP increased over time post-injury which might indicate regeneration over subacute and chronic phase.

In summary, our data suggested that there are patterns of dynamic changes in biomarkers that do occur during the subacute and chronic phase after TBI. Further studies are needed to clarify the pathophysiological role of these proteins during recovery after brain injury.

Materials And Methods

A controlled cortical impact (CCI) was used to model TBI in adult male rats. Different brain tissue regions were collected at various post-CCI time points. Pulverized brain tissue power was lysed with 50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 5 mM EGTA, 1 % Triton X-100, and 1 mM DTT.

Immunoblot analysis and antibodies: Various percentile tris-glycine gels were used for protein separation. Anti-αII-spectrin (Biomol, USA), anti-β-actin (Sigma, USA), LC3 (Novagen, USA) , GFAP (Abcam, USA), CNPase (Fisher Scientifc, USA), MBP (chemicon, USA) were used for immunoblot analysis.

Degradation of alpha II-spectrin following TBI

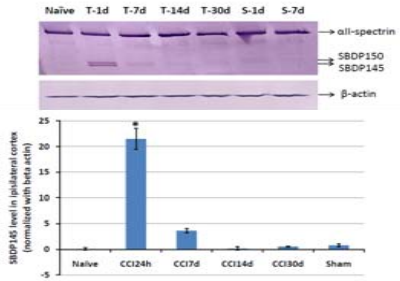

Figure 1: Western blot analysis of αII-spectrin and β-actin expression in rat ipsilateral cortex following traumatic brain injury. The brain samples were collected from naïve, ipsilateral cortex with sham and TBI (2.5 mm impact) 1 day to 30 days after injury. Quantitative analysis of the 145 kDa breakdown product of αII-spectron in ipsilateral cortex were done by densitometry analysis. Data shown are mean ±SEM of at least 3 independent experiments (n=3 to 4). (*p<0.05 compared to sham). A representative blot of various time points is shown.

Autophagy induction following TBI

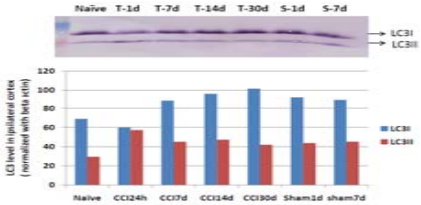

Figure 2: Western blot analysis of LC3 expression in rat ipsilateral cortex following traumatic brain injury. Quantitative analysis of LC3I and LC3II in ipsilateral cortex were done by densitometry analysis. Data shown are mean ±SEM of 2 independent experiments.

Astrocyte biomarker (GFAP) after TBI

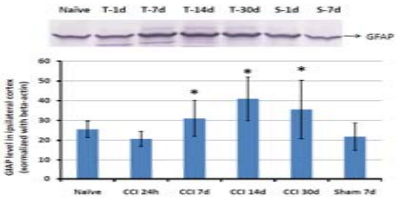

Figure 3: Western blot analysis of GFAP expression in rat ipsilateral cortex following traumatic brain injury. Data shown are mean ±SEM of at least 4 independent experiments (n=4 to 6). (*p<0.05 compared to sham).

Oligodendrocyte biomarker (CNPase) following TBI

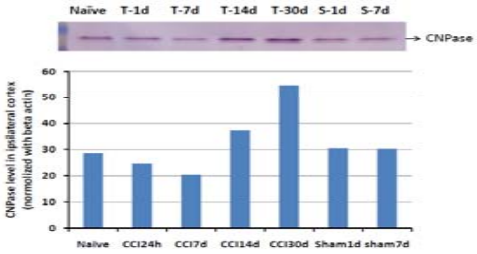

Figure 4: Western blot analysis of CNPase expression in rat ipsilateral cortex following traumatic brain injury. Quantitative analysis of CNPase in ipsilateral cortex were done by densitometry analysis. Data shown are mean ±SEM of 2 independent experiments.

Dysmyelination and regeneration marker (MBP) following TBI

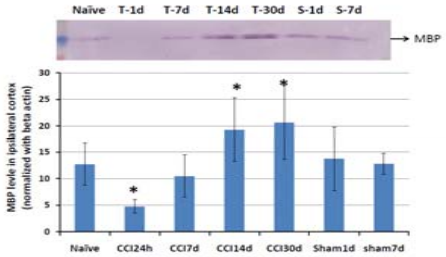

Figure 5: Western blot analysis of MBP expression in rat ipsilateral cortex following traumatic brain injury. Quantitative analysis of MBP in ipsilateral cortex were done by densitometry analysis. Data shown are mean ±SEM of at least 4 independent experiments (n=4 to 5). (*p<0.05 compared to sham).

Conclusions And Future Directions

- Necrosis and autophagy induction occur at acute phase following TBI, as indicated with necrotic cell death marker αII-spectrin breakdown product (SBDP145) and autophagy marker (LC3).
- The expression level of GFAP was dramatically increased following 7 days post-TBI and reached maximal level after 14 days. The level of GFAP stays statistically elevated even after 30 days post-TBI compared to sham. Thus it suggests that GFAP might be a good biomarker for differentiation of acute and sub-acute even chronic brain injury.
- Interesting, the immunoreactivity of oligodendrocyte marker CNPase was found increase by 7 days after TBI, and sustained high level even after 30 days. It showed the potential by using CNPase as a marker for sub-acute and chronic brain injury.
- Myelin basic protein (MBP) decreased significantly 24 h post-TBI and substantial increased after 7 days suggests that dysmyelination occur during early phase of TBI and regeneration induced and sustained during su-bacute and chronic phase of TBI.