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Distinct patterns of expression of traumatic brain injury biomarkers after blast exposure: Role of compromised cell membrane integrity

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Distinct patterns of expression of traumatic brain injury biomarkers after blast exposure: Role of compromised cell membrane integrity

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HIGHLIGHTS

- Repeated blast exposures causes acute decrease in GFAP and *Tau* in brain and plasma.
- GFAP and *Tau* levels increases acutely in the liver and spleen after blast exposure.
- No acute changes in GFAP and *Tau* mRNA levels in the liver after blast exposure.
- The acute changes in GFAP and *Tau* suggest blast-induced cell membrane disruption.

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ABSTRACT

Glial fibrillary acidic protein (GFAP), a protein enriched in astrocytes, and *Tau*, a protein abundant in neuronal microtubules, are being widely studied as biomarkers of brain injury, and persistent severity-dependent increases in brain and blood have been reported. Studies on the acute changes of these proteins after blast exposure are limited. Using a mouse model of closely-coupled repeated blast exposures, we have evaluated acute changes in the levels of GFAP and total *Tau* by Western blotting. Brain levels of GFAP and *Tau* proteins decreased significantly at 6 h and increased considerably at 24 h after repeated blast exposures. Plasma samples showed a similar initial decrease and later increase over this timeframe. This biphasic pattern points to possible absorption or sequestration of these proteins from plasma immediately after repeated blast exposures. Liver and spleen tissue showed significant increases in the levels of GFAP and *Tau* protein at 6 and 24 h post-blast exposures whereas semi-quantitative RT-PCR analysis of liver showed no significant changes in the levels of GFAP or *Tau* mRNAs. These results suggest that blast exposure causes transient changes in cell membrane integrity in multiple organs leading to abnormal migration of proteins from the tissues to the plasma and *vice versa*. This transient changes in cell membrane permeability and subsequent bidirectional movement of molecules may contribute to the pathophysiology of TBI and polytrauma after blast exposure.

1. Introduction

The incidence of traumatic brain injury (TBI) increased tremendously during the recent wars and exposure to blast from improvised explosive devices has been reported as the major cause of battlefield TBI and associated disabilities in service members [14]. One of the major differences between blast-induced TBI and other closed head or penetrating brain injuries is that blast exposure concomitantly injures other organs of the body, especially air filled organs, resulting in polytrauma. Military personnel are subject to both high intensity single or low intensity repeated blast exposures, and we have previously reported that the severity of brain injury increases with number of blast exposures [27].

Identification of sensitive and specific biomarkers of TBI is potentially useful for the diagnosis of injury and evaluation of the efficacy of therapies. Although no biomarkers unique to blast-induced TBI have been reported in clinical or pre-clinical blast exposure studies to date, several brain proteins that are being

Abbreviations: TBI, traumatic brain injury; GFAP, glial fibrillary acidic protein; BOP, blast overpressure; CSF, cerebrospinal fluid; mRNA, messenger ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction.

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widely evaluated as biomarkers of other forms of brain injury have also been considered as biomarkers of blast TBI. Notably, glial fibrillary acidic protein (GFAP), a protein enriched in astrocytes, and *Tau*, a protein abundant in neuronal microtubules has been monitored after various forms of brain injury including blast-induced TBI [4,7,10,22,24,25].

Changes in blood and cerebrospinal fluid (CSF) levels of GFAP have been widely studied as sensitive biomarkers for the diagnosis TBI in humans [5,9,13,19,21,26,28] and significant up-regulation of GFAP levels in multiple regions of the brain has also been reported at different intervals after blast exposure in animal models of blast-induced TBI [4,7,10,24]. In all these pre-clinical studies, the changes in GFAP were evaluated after 24 h post-exposure. Changes in GFAP levels in the brain or body fluids immediately after single blast exposure have not been well documented. Besides, there are no studies addressing the effects on GFAP levels in the brain or blood after repeated blast exposures.

Apart from GFAP, up-regulation of total and phosphorylated *Tau* proteins have also been shown in the brain, CSF and blood of patients with different forms of TBI, and hence these proteins have been proposed as reliable biomarkers of outcome after TBI [11,15,20,23]. Total and phosphorylated *Tau* proteins in the brain were significantly increased in different animal models of TBI [7,11,22,25]. Limited studies have been carried out to assess the changes in total or phosphorylated *Tau* proteins in the brain or body fluids after blast exposure and there are no such studies after repeated blast exposures. Similar to studies evaluated GFAP levels, the differential expression of *Tau* protein was determined only at 24 h or later in single blast exposure and no studies so far investigated the acute changes in *Tau* proteins after repeated blast exposures.

In the present study, we assessed immediate changes in GFAP and total *Tau* protein in the brains and plasma of mice after closely coupled repeated blast overpressure exposures in a shock tube as described earlier [27]. Western blotting with specific antibodies was used to assess the levels of these potential blast injury biomarkers. Our results after repeated blast exposures showed an initial decrease and later increase in the levels of both GFAP and total *Tau* proteins in the brain and plasma. We further explored the potential mechanism of the contrasting changes in the two biomarkers of TBI after repeated blast exposures.

2. Materials and methods

2.1. Animals and blast exposures

All animal experiments were conducted in accordance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC Publication 1996 edition). The animal protocol was approved by Institutional Animal Care and Use Committee, Walter Reed Army Institute of Research. C57BL/6J male mice (8–10 weeks old) that weighed between 21 and 26 g (Jackson Laboratory, Bar Harbor, ME) were used in this study. A compressed air-driven shock tube described earlier [12,27] was used for repeated blast exposures. Mice were anesthetized with 4% isoflurane gas (O_2 flow rate 1.5 L/min) for 8 min and restrained in the prone position with a tautly-drawn net to minimize the movements during blast exposure. Animals were subjected to three blast overpressure (BOP) exposures (21 psi) separated by 1 and 30 min as described earlier [28]. Blood plasma, brain, liver and spleen were collected after euthanasia at 6 or 24 h post-blast and frozen immediately. Cerebellum, which showed significant injury after blast exposure in previous studies [27] was dissected out immediately before freezing the brain.

2.2. Western blot analysis of tissue homogenates

Tissue homogenates (20%, w/v) were prepared in tissue protein extraction buffer (Pierce Chemical Co., Rockford, IL) containing protease and phosphatase inhibitor cocktails (Sigma–Aldrich, St. Louis, MO) using an ultrasonic homogenizer. The homogenates were centrifuged at $5000 \times g$ for 5 min at 4 °C and 2 μl each of supernatants were used for Western blotting. Rabbit polyclonal antibodies against GFAP and total *Tau* protein were obtained from Abcam (Cambridge, MA) and Santa Cruz Biotechnologies (Santa Cruz, CA), respectively. GFAP antibody was used at a dilution of 1:40,000 and an antibody to total *Tau* proteins was used at 1:1000 dilution. Secondary antibody labeled with horse-radish peroxidase (HRP) was also purchased from Santa Cruz Biotechnology and used at a dilution of 1:2500. A mouse monoclonal antibody to β-actin conjugated with HRP (Sigma–Aldrich, St. Louis, MO) was used as gel loading control at a dilution of 1:40,000. SDS–polyacrylamide gel electrophoresis and Western blotting analysis of protein extracts from cerebellum, liver and spleen was carried out as described earlier [3]. After Western blotting, the protein bands were detected using ECL-Plus Western blot detection reagent (GE Healthcare, Piscataway, NJ) and the chemiluminescence was imaged in an AlphaImage reader (Cell Biosciences, Santa Clara, CA). The band intensity was measured by densitometry using AlphaView software (Cell Biosciences, Santa Clara, CA).

2.3. Western blotting of GFAP and tau proteins in the plasma

Plasma was purified by removing albumins and globulins using ProteoExtract Albumin/IgG removal kit from EMD-Millipore Corporation (Chicago, IL) according to manufacturer's instructions. Briefly, 850 μl of binding buffer was allowed to pass through the column by gravity-flow. Plasma (35 μl) was diluted 10-fold with binding buffer, allowed to pass through the column and the flow-through was collected. The unbound proteins were also collected by passing 650 μl of binding buffer though the column twice. The collected flow-through fractions were pooled and concentrated using a 3000 MW cut off VIVASPIN 500 centrifugal filters (Sartorius Stedim, Bohemia, NY) according to manufacturer's instructions. Concentrated albumin and globulin free fractions corresponding to 5 μl of original plasma were used for Western blotting as described above.

2.4. Semi-quantitative RT-PCR analysis of liver tissue

Frozen mouse liver tissue was thawed and homogenized by pipetting with 1 ml of TRIzol reagent (Life Technologies, Grand Island, NY). Total RNA was isolated according to manufacturer's protocol. For cDNA synthesis, total RNA (1 μg) was subjected to reverse transcription in a reaction mixture containing dNTPs (0.5 mM), random hexamer primer (0.01 μg/μl), and 40 U of RNase inhibitor (Thermo Scientific, Waltham, MA) and RNasin (Promega Corporation, Madison, WI) for 45 min at 50 °C. Using premix PCR mixture (Thermo Scientific, Waltham, MA), PCR was performed in the presence of 0.33 μM of gene specific primers and 0.5 μl of cDNA in thermal cycler as follows; 95 °C × 1 min, followed by 35 cycles of 95 °C × 30 s, 61 °C × 30 s, 72 °C × 30 s, subsequently 72 °C × 5 min. Primers for GFAP were 5′-CTGGCTGCGTATAGACAGGA-3′ as forward, 5′-GAACTGGATCTCCTCCTCCA-3′ as reverse, and primers for *Tau* were 5′-GTGGAGGCAGTGTGCAAATA-3′ as forward, 5′-GCCAATCTTCGACTGGACTC-3′ as reverse. Aliquots were loaded and electrophoresed in 2% agarose gel. PCR product was visualized with ethidium bromide under UV illuminator and the image was captured using GeneSnap software (Syngene, Frederick, MD). Brain tissue from sham control mice was used as positive control.

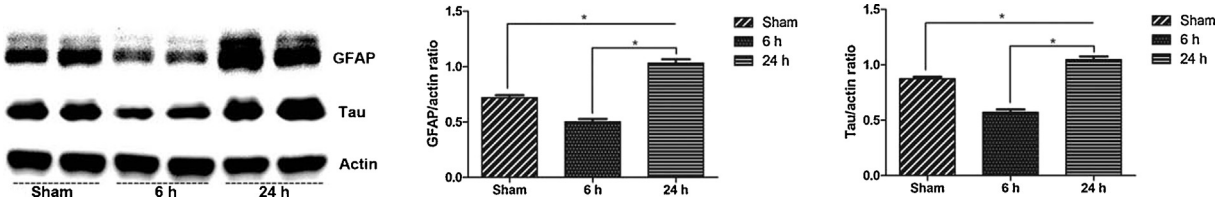

Fig. 1. Western blot analysis of the cerebellum showing the immediate decrease and later increase in the levels of GFAP and *Tau* proteins after repeated blast exposures. Representative blot from two out of four animals in each group is presented. Densitometry analysis was carried out as described in Section 2. $^{*}p < 0.01$ ($n = 4$).

2.5. Statistical analysis

Statistical analysis was carried out by analysis of variance (ANOVA) using SAS software version 9.3. Values were expressed as mean ± standard deviation (SD). A p value less than 0.01 was considered significant.

3. Results

3.1. Changes in GFAP and total Tau protein expression in the brain after blast exposure

Western blot analyses of the cerebellum revealed biphasic time-dependent changes in the expression of both GFAP and total *Tau* proteins after repeated blast exposures (Fig. 1). The levels of these proteins decreased significantly at 6 h and increased appreciably at 24 h after repeated blast exposures. Densitometry analysis indicated that the ratios of GFAP to actin as well as total *Tau* to actin were significantly reduced at 6 h and increased considerably at 24 h after repeated blast exposures.

3.2. Levels of GFAP and total Tau proteins in the plasma after blast exposure

Similar to the brain, biphasic changes in the levels of both GFAP and total *Tau* proteins were recorded in the plasma after repeated blast exposures (Fig. 2). Densitometry of the Western blot showed that plasma levels of GFAP as well as total *Tau* proteins were significantly reduced at 6 h and increased at 24 h after repeated blast exposures.

3.3. Levels of GFAP and total Tau proteins in the liver and spleen after blast exposure

GFAP and total *Tau* proteins were readily detected in the liver and spleen of sham control mice. Densitometry analysis and the ratios of GFAP to actin as well as total *Tau* protein to actin indicated a significant increase in the levels of both GFAP and total *Tau* proteins in the liver and spleen at 6 and 24 h after blast repeated exposures (Fig. 3). Compared to 6 h, the increase in the levels of both GFAP and total *Tau* proteins at 24 h was marginal and was not statistically significant in both tissues.

3.4. Expression of GFAP and Tau mRNA levels in the liver after blast exposure

To investigate whether the increase in GFAP and *Tau* proteins in the liver is due to increased synthesis, RT-PCR of liver tissue mRNA was performed and showed the presence of products with the same base pairs corresponding to GFAP and *Tau* proteins in the brain. Semi-quantitative RT-PCR analysis of liver tissues indicated that the levels of GFAP or *Tau* mRNAs were not increased significantly at 6 or 24 h after repeated blast exposures (Fig. 4).

4. Discussion

Our results showed for the first time that repeated blast exposures caused pronounced biphasic changes in two well-known biomarkers of TBI in the brain and plasma. In the brain and plasma, the levels of GFAP and total *Tau* proteins decreased significantly at 6 h after repeated blast exposures, whereas their levels were increased at 24 h post-blast exposures. The increase in the expression of GFAP at 24 h after blast exposure is in agreement with the previous preclinical studies [4,24]. To our knowledge, no studies have established this acute change in GFAP in the brain or plasma after single or repeated blast exposures. In the case of *Tau* protein, measured changes in total or phosphorylated *Tau* have been restricted to several days after single blast exposure [7,10].

The significant decrease in GFAP and Tau proteins in the brain at 6 h after repeated blast exposures was an unanticipated and somewhat paradoxical result. Since the turnover of GFAP and *Tau* proteins in the brain is very slow [6,16], the acute decrease in GFAP/Tau in the brain after blast exposure can most likely be interpreted as a blast-induced rapid disruption of glial/neuronal cell membranes and protein leakage across a disrupted blood–brain barrier into the circulation. However, Western blotting of plasma showed a similar significant decrease in the levels of both the proteins at 6 h after repeated blast exposures (Fig. 2). Parallel decreases of both putative biomarkers in brain and plasma shortly after blast exposure points to their rapid redistribution or elimination from the blood during this acute timeframe.

Using an *in vitro* model of blast-induced TBI, it has recently been shown that blast exposure causes transient disruption of neuronal cell membrane integrity leading to bidirectional movement of molecules across the cell membrane [1]. Rapid disruption of cell membrane integrity of liver cells and muscle fibers and the

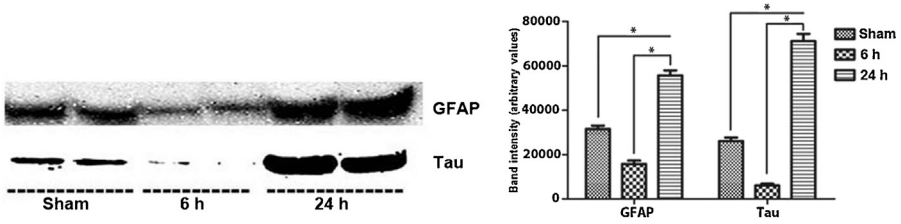

Fig. 2. Western blot analysis of plasma showing the immediate decrease and later increase in the levels of GFAP and *Tau* proteins after repeated blast exposures. Representative blot from two out of four animals in each group is presented. Densitometry analysis was carried out as described in Section 2.$^{*}p < 0.01$ ($n = 4$).

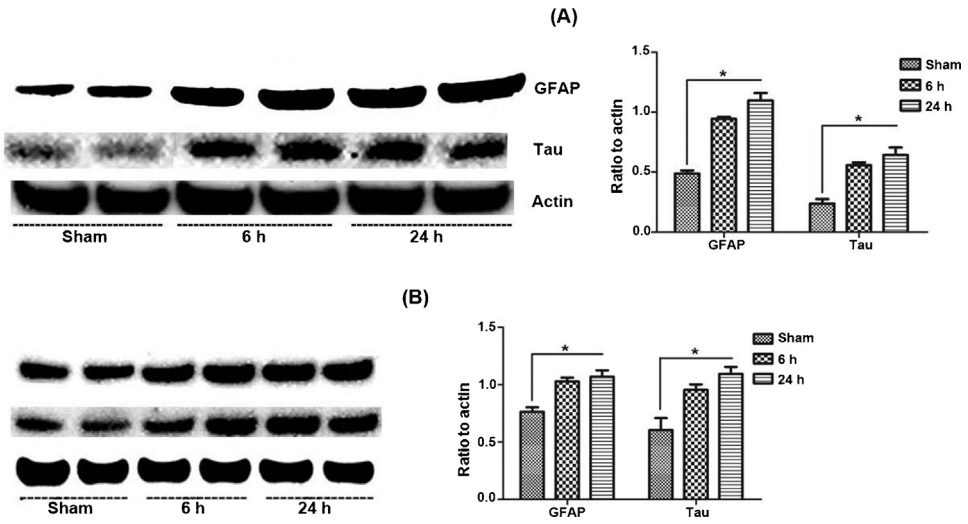

Fig. 3. Western blot analysis of liver (A) and spleen (B) showing the levels of GFAP and *Tau* proteins at 6 and 24 h after repeated blast exposures. Representative blots from two out of four animals in each group for liver and spleen are presented. Densitometry analysis was carried out as described in Section 2.*$p < 0.01$ ($n = 4$).

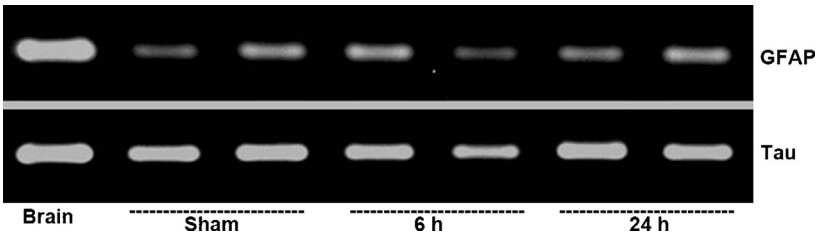

Fig. 4. Semi-quantitative RT-PCR analysis of liver showing the mRNA levels of GFAP and Tau proteins. Total RNA isolated from the brain of a sham control mice was used as positive control. RT-PCR was carried out as described in the methods section. Representative figure from two out of four animals in each group is presented.

subsequent release of organ specific cellular proteins into the circulation after repeated blast exposures have also been reported [2]. Collectively, these data suggest that blast exposure affects the cell membrane integrity of brain and peripheral organs which can lead to widespread bidirectional passage of molecules. The various potential mechanisms of cell membrane disruption after blast exposure have been described in detail by Nakagawa et al. [18].

The parallel decreased levels of GFAP/*Tau* proteins in the brain and plasma at 6 h after repeated blast exposures suggests a possible shift and accumulation of these proteins in peripheral organs. Liver is one of the largest organs of the body and it showed blast overpressure-dependent release of enzyme to circulation, revealing cell membrane disruption [2]. Based upon earlier studies of liver after blast exposure and the expression of GFAP and *Tau* proteins in this organ [2,8,17], we investigated blast-induced changes in the levels of these proteins in the liver. We also examined the spleen, which is a primary component of the reticuloendothelial system where the blood is being processed. Increased levels of GFAP and *Tau* in both the liver and spleen coincided with their simultaneously decreased levels in the brain and plasma at 6 h after repeated blast exposures. To investigate whether their increased levels resulted from locally increased synthesis, we measured the mRNA levels of GFAP and *Tau* proteins in the liver. The semi-quantitative RT-PCR analysis data showed no significant increase in the mRNA levels of GFAP and *Tau* proteins in the liver. These data reinforce the conclusion that these proteins transiently accumulate in these organs following passage from the circulation after repeated blast exposures.

Although GFAP and Tau proteins remained elevated in the liver and spleen after blast exposure, there was no significant difference in the levels at 24 h compared to 6 h, even though the levels of these proteins increased substantially in the plasma at 24 h after the blast exposure. Restoration of vascular, liver, and spleen cell membrane integrity may have prevented further passage of these proteins in these organs. Previous studies using *in vitro* and *in vivo* models of blast-induced TBI indicate that the cell membrane disruption after blast exposure is transient and the restoration of cell membrane integrity take place rapidly [1,2].

A potential pathophysiological consequence of transient disruption of cell membrane integrity after blast exposure is the transport of foreign molecules, including proteins, which can enter various cells of peripheral organs and brain from the blood circulation and may remain intracellularly for longer time leading to chronic pathological changes. Furthermore, the essential molecules rapidly released from the cells due to transient disruption of cell membrane after blast exposure can also affect cellular homeostasis. Thus, the rapid abnormal bidirectional movement of molecules in the brain and peripheral organs immediately after blast exposure may have the possibility to trigger long-term consequences, a potential mechanism contributing to blast-induced polytrauma. These time-dependent bidirectional changes also point to a fundamental issue complicating and potentially limiting the utility of these circulating proteins as acute biomarkers of blast injury.

Disclosure

The contents, opinions and assertions contained herein are private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense. The authors report no conflict of interest.

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References

[1] P. Arun, R. Abu-Taleb, M. Valiyaveettil, Y. Wang, J.B. Long, M.P. Nambiar, Transient changes in neuronal cell membrane permeability after blast exposure, NeuroReport 23 (2012) 342–346.
[2] P. Arun, S. Oguntayo, Y. Alamneh, C. Honnold, Y. Wang, M. Valiyaveettil, J.B. Long, M.P. Nambiar, Rapid release of tissue enzymes into blood after blast exposure: potential use as biological dosimeters, PLoS ONE 7 (2012) e33798.
[3] P. Arun, M. Valiyaveettil, L. Biggemann, Y. Alamneh, Y. Wei, S. Oguntayo, Y. Wang, J.B. Long, M.P. Nambiar, Modulation of hearing related proteins in the brain and inner ear following repeated blast exposures, Interventional Medicine and Applied Science 4 (2012) 125–131.
[4] I. Cernak, A.C. Merkle, V.E. Koliatsos, J.M. Bilik, Q.T. Luong, T.M. Mahota, L. Xu, N. Slack, D. Windle, F.A. Ahmed, The pathobiology of blast injuries and blast-induced neurotrauma as identified using a new experimental model of injury in mice, Neurobiology of Disease 41 (2011) 538–551.
[5] E. Czeiter, S. Mondello, N. Kovacs, J. Sandor, A. Gabrielli, K. Schmid, F. Tortella, K.K. Wang, R.L. Hayes, P. Barzo, E. Ezer, T. Doczi, A. Buki, Brain injury biomarkers may improve the predictive power of the IMPACT outcome calculator, Journal of Neurotrauma 29 (2012) 1770–1778.
[6] S.J.DeArmond, Y.L. Lee, H.A. Kretzschmar, L.F. Eng, Turnover of glial filaments in mouse spinal cord, Journal of Neurochemistry 47 (1986) 1749–1753.
[7] L.E. Goldstein, A.M. Fisher, C.A. Tagge, X.L. Zhang, L. Velisek, J.A. Sullivan, C. Upreti, J.M. Kracht, M. Ericsson, M.W. Wojnarowicz, C.J. Goletiani, G.M. Maglakelidze, N. Casey, J.A. Moncaster, O. Minaeva, R.D. Moir, C.J. Nowinski, R.A. Stern, R.C. Cantu, J. Geiling, J.K. Blusztajn, B.L. Wolozin, T. Ikezu, T.D. Stein, A.E. Budson, N.W. Kowall, D. Chargin, A. Sharon, S. Saman, G.F. Hall, W.C. Moss, R.O. Cleveland, R.E. Tanzi, P.K. Stanton, A.C. McKee, Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model, Science Translational Medicine 4 (2012) 134ra60.
[8] Y. Gu, F. Oyama, Y. Ihara, Tau is widely expressed in rat tissues, Journal of Neurochemistry 67 (1996) 1235–1244.
[9] M. Honda, R. Tsuruta, T. Kaneko, S. Kasaoka, T. Yagi, M. Todani, M. Fujita, T. Izumi, T. Maekawa, Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase, Journal of Trauma 69 (2010) 104–109.
[10] E. Kovesdi, A.B. Gyorgy, S.K. Kwon, D.L. Wingo, A. Kamnaksh, J.B. Long, C.E. Kasper, D.V. Agoston, The effect of enriched environment on the outcome of traumatic brain injury; a behavioral, proteomics, and histological study, Frontiers in Neuroscience 5 (2011) 42.
[11] P.C. Liliang, C.L. Liang, H.C. Weng, K. Lu, K.W. Wang, H.J. Chen, J.H. Chuang, Tau proteins in serum predict outcome after severe traumatic brain injury, Journal of Surgical Research 160 (2010) 302–307.
[12] J.B. Long, T.L. Bentley, K.A. Wessner, C. Cerone, S. Sweeney, R.A. Bauman, Blast overpressure in rats: recreating a battlefield injury in the laboratory, Journal of Neurotrauma 26 (2009) 827–840.
[13] K.M. Lumpkins, G.V. Bochicchio, K. Keledjian, J.M. Simard, M. McCunn, T. Scalea, Glial fibrillary acidic protein is highly correlated with brain injury, Journal of Trauma 65 (2008) 778–782.
[14] J. Magnuson, F. Leonessa, G.S. Ling, Neuropathology of explosive blast traumatic brain injury, Current Neurology and Neuroscience Reports 12 (2012) 570–579.
[15] N. Marklund, K. Blennow, H. Zetterberg, E. Ronne-Engstrom, P. Enblad, L. Hillered, Monitoring of brain interstitial total tau and beta amyloid proteins by microdialysis in patients with traumatic brain injury, Journal of Neurosurgery 110 (2009) 1227–1237.
[16] J.Morales-Corraliza, M.J. Mazzella, J.D. Berger, N.S. Diaz, J.H. Choi, E. Levy, Y. Matsuoka, E. Planel, P.M. Mathews, In vivo turnover of tau and APP metabolites in the brains of wild-type and Tg2576 mice: greater stability of sAPP in the beta-amyloid depositing mice, PLoS ONE 4 (2009) e7134.
[17] S. Morini, S. Carotti, G. Carpino, A. Franchitto, S.G. Corradini, M. Merli, E. Gaudio, GFAP expression in the liver as an early marker of stellate cells activation, Italian Journal of Anatomy and Embryology 110 (2005) 193–207.
[18] A. Nakagawa, G.T. Manley, A.D. Gean, K. Ohtani, R. Armonda, A. Tsukamoto, H. Yamamoto, K. Takayama, T. Tominaga, Mechanisms of primary blast-induced traumatic brain injury: insights from shock-wave research, Journal of Neurotrauma 28 (2011) 1101–1119.
[19] K. Nylen, M. Ost, L.Z. Csajbok, I. Nilsson, K. Blennow, B. Nellgard, L. Rosengren, Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome, Journal of the Neurological Sciences 240 (2006) 85–91.
[20] M. Ost, K. Nylen, L. Csajbok, A.O. Ohrfelt, M. Tullberg, C. Wikkelso, P. Nellgard, L. Rosengren, K. Blennow, B. Nellgard, Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury, Neurology 67 (2006) 1600–1604.
[21] L.E. Pelinka, A. Kroepfl, R. Schmidhammer, M. Krenn, W. Buchinger, H. Redl, A. Raabe, Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma, Journal of Trauma 57 (2004) 1006–1012.
[22] E. Rostami, J. Davidsson, K.C. Ng, J. Lu, A. Gyorgy, J. Walker, D. Wingo, S. Plantman, B.M. Bellander, D.V. Agoston, M. Risling, A model for mild traumatic brain injury that induces limited transient memory impairment and increased levels of axon related serum biomarkers, Frontiers in Neurology 3 (2012) 115.
[23] C. Smith, D.I. Graham, L.S. Murray, J.A. Nicoll, Tau immunohistochemistry in acute brain injury, Neuropathology and Applied Neurobiology 29 (2003) 496–502.
[24] S.I. Svetlov, V. Prima, D.R. Kirk, H. Gutierrez, K.C. Curley, R.L. Hayes, K.K. Wang, Morphologic and biochemical characterization of brain injury in a model of controlled blast overpressure exposure, Journal of Trauma 69 (2010) 795–804.
[25] H.T. Tran, L. Sanchez, T.J. Esparza, D.L. Brody, Distinct temporal and anatomical distributions of amyloid-beta and tau abnormalities following controlled cortical impact in transgenic mice, PLoS ONE 6 (2011) e25475.
[26] P.E. Vos, B. Jacobs, T.M. Andriessen, K.J. Lamers, G.F. Borm, T. Beems, M. Edwards, C.F. Rosmalen, J.L. Vissers, GFAP S100B are biomarkers of traumatic brain injury: an observational cohort study, Neurology 75 (2010) 1786–1793.
[27] Wang, Y. Wei, S. Oguntayo, W. Wilkins, P. Arun, M. Valiyaveettil, J. Song, J. Long, M.P. Nambiar, Tightly coupled repetitive blast-induced traumatic brain injury: development and characterization in mice, Journal of Neurotrauma 28 (2011) 2171–2183.
[28] J. Zurek, M. Fedora, The usefulness of S100B, NSE GFAP, NF-H, secretagogin and Hsp70 as a predictive biomarker of outcome in children with traumatic brain injury, Acta Neurochirurgica (Wien) 154 (2012) 93–103.