



REVIEW ARTICLE

Pathophysiological Findings of Selective Vulnerability in the Hippocampus After Traumatic Brain Injury

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ARTICLE INFO

Article history:

Received: Apr 12, 2010

Revised: Sep 25, 2010

Accepted: Nov 11, 2010

KEY WORDS:

cell signaling;
hippocampus;
memory disturbance;
pathophysiology;
traumatic brain injury;
vulnerability

Posttraumatic amnesia is a common symptom after traumatic brain injury (TBI), and it may be related to hippocampal dysfunction. However, the mechanisms underlying the secondary or delayed cell death in the hippocampus after TBI have been poorly understood. In this article, we review the clinical and experimental evidence of hippocampal damage after TBI. Recent data suggesting that TBI may be both an inflammatory and/or a neurodegenerative disease are also presented. Further investigations are, therefore, necessary to better understand hippocampal damage and delineate the best practice strategies needed to improve the patient outcomes after TBI.

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Traumatic brain injury (TBI) is a leading cause of morbidity and mortality worldwide, particularly among young people.^{1,2} The majority of these cases are classified as mild TBI, regardless of severity, and a significant number of patients report some degree of neurobehavioral sequelae after TBI. The incidence of mild TBI patients with persistent symptoms is approximately 27/100,000, estimated to be 15% of mild TBI incidence, which is equal to the incidence of Parkinson's disease.³ Particularly, memory deficits were found in 50% of patients with mild TBI and 90% of patients with moderate TBI.⁴ Posttraumatic amnesia is a common symptom following TBI, and may be related to hippocampal dysfunction.⁵ Patients with mild TBI and persistent postconcussive symptoms have a high incidence of medial temporal lobe injury.⁶ We herein present a case of a head injury caused by a traffic accident. A 55-year-old male suffered a simple head injury in a head-on car collision. On admission, he was agitated. An initial computed tomography (CT) scan showed diffuse injury (Figure 1A). The minimal state examination score was 21 out of 30, which showing cognitive dysfunction, particularly, recent memory disturbance persisted. An MRI analysis performed 36 hours after the initial trauma revealed bilateral hippocampal damage (Figure 1B and 1C). Pathological examinations in humans and in animal models after

TBI demonstrate hippocampal neuronal damage, which may contribute to cognitive impairment.

Magnetic resonance imaging (MRI) volumetric studies showed that predicting the association between TBI and premature loss of brain parenchyma is important in determining the most serious injuries. Blatter et al⁷ demonstrated that the predictable time-dependent atrophic changes occurring after TBI could be quantified using volumetric MRI. The authors showed a high to moderate correlation between atrophy and cognitive outcome, and regional hippocampal volume reduction and temporal horn enlargement are correlated with decreased verbal memory function.^{8,9} The hippocampus and temporal horn volumes were inversely correlated in the group with TBI, suggesting a differential relationship of these structures in TBI patients as compared with control subjects. MRI volumetric studies measuring the volume of the temporal horn and hippocampus may be predictive of the eventual cognitive outcome. Tomaiulo et al¹⁰ demonstrated that in severe TBI without obvious neuroradiological lesions, there was a clear hippocampal, fornix, and callosal volume reduction. The coma duration predicted the callosal volume reduction, which may be a marker of the severity of axonal loss. This relationship with memory performance may reflect the diffuse nature of the damage, and may lead to the disruption of neuronal circuits at multiple levels and the progressive neuronal degeneration occurring in TBI. Serra-Grabulosa et al¹¹ investigated residual brain damage in subjects who suffered severe TBI in childhood, and its relationship with declarative memory impairment. The TBI patients performed significantly worse than controls

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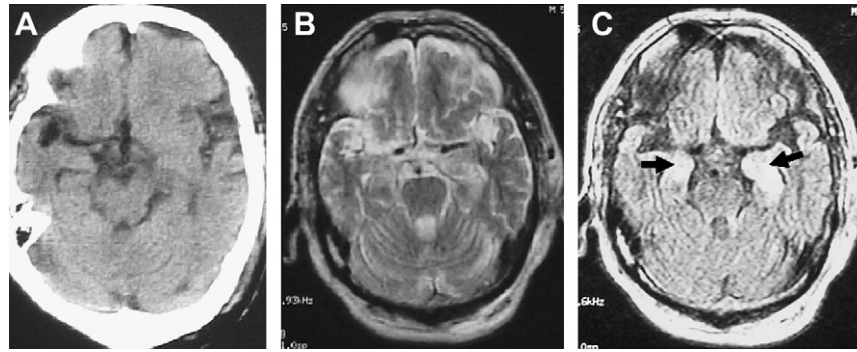


Figure 1 A 55-yr-old man suffered a simple head injury in a head-on car collision. On admission, he was agitated. (A) An initial computed tomography scan showed diffuse injury. The mini-mental state examination score was 21 out of 30. Cognitive dysfunction persisted, particularly, recent memory disturbance. (B) T2-weighted and (C) Fluid-attenuated inversion recovery magnetic resonance imaging performed 36 hr after trauma showed bilateral hippocampal damage (arrow head).

subjects in both verbal and visual memory. These patients presented with decreased white matter volume and increased cerebrospinal fluid (CSF) volume. The hippocampus was reduced, but not the caudate nuclei. The memory performance correlated with the CSF volume. Hippocampal atrophy, white matter loss, and memory impairment persisted until adolescence. Memory sequelae were related more to diffuse brain injury, as revealed by the MRI findings of increased CSF volume, than to hippocampal injury.

Since abnormalities of the hippocampus are associated with memory dysfunction, many experimental studies investigated the effects of TBI on hippocampal structure and function in a rat model. In the review by Lighthall et al¹² regarding experimental TBI models, the authors described and characterized the pathophysiologic changes using a fluid percussion (FP) method and a controlled cortical impact (CI) technique. The new models were compared to prior experimental brain injury techniques in relation to ongoing physical and analytical modeling used in automotive safety research. They concluded that although the FP technique was the most widely used method for producing brain injury, it was useful for producing graded injury responses systemically and centrally. Chen et al¹³ described the characterization of an experimental model of closed head injury in a mouse model. The posttraumatic accumulation of cerebral edema, the disruption of the blood-brain barrier, histopathology, motor and cognitive functions were investigated up to 30 days following closed head (CH) injury. In the hippocampus, overt neuronal cell death was observed only in the CA3 area at 7 days following CH injury. Thompson et al¹⁴ comprehensively reviewed journals about the lateral fluid percussion (LFP) model in small animal species with particular emphasis on its validity, clinical relevance, and reliability in producing both focal and diffuse brain injury. The LFP model has also been used extensively to evaluate potential therapeutic interventions, including resuscitation, pharmacologic therapies, transplantation, and other neuroprotective and neurodegenerative strategies. The authors concluded that the LFP brain injury model was an appropriate tool to study the cellular and mechanistic aspects of human TBI that cannot be addressed in a clinical setting. In addition, Dixon et al¹⁵ characterized a new LFP model of experimental brain injury to systematically examine the physiologic and histopathologic responses in rats at two levels of injury severity. The data suggested that the distributed nature of the LFP injury induced pathology, and dysfunction reflected a diffuse mechanical loading of the brain surface. The model provided reproducible effects that were useful in the study of closed head injury. Lighthall¹⁶ described a new experimental model of mechanical brain injury using a stroke-constrained pneumatic impactor. Using this injury model, the spectrum of anatomic injury and systemic physiologic responses closely resembled aspects of closed head injury observed clinically. Graded cortical injury was

produced by precisely controlled brain deformations, thereby determining the influence of contact velocity and the level of deformation on the anatomic and functional severity of brain injury. These reports suggested that the modified injury model could reproduce the posttraumatic sequelae observed in rats and that some of the data obtained in this model were essentially similar to those observed in human brain injuries.

Hicks et al¹⁷ systematically characterized the pattern of neuronal injury at sequential time points to identify the selectively vulnerable regions and to determine the temporal contribution of primary and delayed neuropathological events following LFP brain injury in rats. The frequency of injured neurons was greatest in the ipsilateral cortex, hippocampus, and thalamus, and a visible loss of Nissl-stained neurons was observed in these regions starting at 12 hours after injury. Those findings suggested that LFP brain injury in the rat produced a combination of focal cortical contusion and diffuse subcortical neuronal injury, which was present within minutes of the impact, progressed to neuronal loss by 12 hours, and did not markedly expand into other brain regions with survival periods of up to 7 days. Several experimental studies have suggested that a selective vulnerability to TBI was observed in hippocampal CA3 neurons.^{17,18} Immonen et al¹⁹ suggested that the assessment of early quantitative MRI changes in the hippocampus and in the perifocal area might help to predict the long-term outcome after experimental TBI.

Memory dysfunction following head injury is a prominent feature in humans, yet the biological basis of this abnormality remains unknown. The CA3 area is critical for cognitive functions related to memory recall through pattern completion.²⁰ CA3 *N*-methyl-D-aspartate (NMDA) receptor knockout mice were impaired in retrieving spatial reference memory in the Morris water maze when presented with a fraction of the original cue. Significant memory deficits were associated with neuronal cell death in the bilateral hippocampus following TBI in the CA3 and dentate hilus lesions.^{13,21–23} Smith et al²⁴ demonstrated that memory dysfunction could persist for at least 2 weeks following LFP brain injury, and that it was associated with a selective bilateral loss of neurons in the dentate hilar region of the hippocampus. Given the important role of the hippocampus in memory, those observations provided a potential anatomic substrate to explain, in part, the development of posttraumatic memory deficits. Hamm et al²⁵ examined the effects of brain injury on spatial learning and memory using the Morris water maze task. The results revealed that injured rats exhibited significant deficits in maze performance at both testing intervals. Since the Morris water maze task is particularly sensitive to hippocampal dysfunction, the results of that experiment supported the hypothesis that the hippocampus was preferentially vulnerable to damage after TBI. Those results also demonstrated that CH brain injury produced enduring cognitive deficits analogous to those observed after human brain injury. Witgen et al²⁶ showed that

the cognitive deficits persisted in patients who survived TBI. LFP brain injury in the mouse, a model of human TBI, resulted in hippocampal dependent cognitive impairment, similar to retrograde amnesia often associated with TBI. The authors evaluated regional neuronal loss, regional hippocampal excitability and inhibitory synaptic transmission to identify potential substrates of the cognitive impairment. The study revealed for the first time that brain injury selectively disrupted hippocampal function by causing uniform neuronal loss, inhibitory synaptic dysfunction, and regional, but opposing, shifts in circuit excitability. These changes may contribute to the cognitive impairments that resulted from brain injury.

Nawashiro et al.²⁷ demonstrated the selective vulnerability of hippocampal CA3 neurons to hypoxia after mild concussion in rats. In addition, Forbes et al.²⁸ reported that aggressive hyperventilation early after TBI augmented CA3 hippocampal neuronal death. The data indicated that CA3 hippocampal neurons were selectively vulnerable to the effects of hyperventilation after TBI. The injured neurons were shown as Nissl-stained dark neurons. Ooigawa et al.²⁹ studied the fate of Nissl-stained dark neurons after TBI. In the hippocampus the number of dead neurons was approximately the same number as that of the Nissl-stained dark neurons, and most dark neurons showed an increased phosphorylated ERK immunoreactivity. The data suggested that not all Nissl-stained dark neurons inevitably died after TBI. Lowenstein et al.³⁰ showed that neurons of the dentate hilus were vulnerable to a brief, unilateral impact to the extradural surface of the brain using a model of fluid percussion injury (FPI). This neuronal loss was highly selective since the adjacent dentate granule and pyramidal neurons appeared relatively unaffected. The presence of abnormal hyperexcitability correlated with the loss of hilar neurons. Thus, a momentary impact to the surface of the brain could cause selective, bilateral hippocampal injury with associated abnormalities in dentate gyrus physiology. Tran et al.³¹ explored the morphological and physiological response of the contralateral hippocampus to LFP injury. In the contralateral hippocampus, the transient decrease in the number of healthy neurons was observed. The cognitive recovery observed after human TBI possibly stems from differential injury exposure and the time course of recovery between the homologous regions of the two hemispheres. Despite intensive investigational studies, the neurobiological basis of epilepsy and persistent memory impairment following TBI remains unknown. In particular, the mechanism of posttraumatic selective vulnerability of hippocampal CA3 neurons has not yet been fully elucidated.

Numerous experimental studies have been reported to evaluate the pathophysiological findings following TBI. Abegg et al.³² obtained whole-cell patch clamp recordings from pyramidal cells in hippocampal slice cultures while mechanically lesioning the CA3 area. Electrophysiological analysis revealed that TBI massively increased excitatory and inhibitory synaptic activities in the entire CA3 region. Matsushita et al.³³ suggested that TBI induced neuronal depolarization and excessive excitatory neurotransmitter release, which enhanced glutamate toxicity and led to an increase in intracellular calcium levels. The authors examined the extracellular concentrations of glutamate during hypoxia using Ringer solution containing a high potassium level, and using a microdialysis probe, which was stereotactically inserted into the hippocampus. Consequently, the extracellular concentration of potassium played a role in the mechanism of increased neuronal vulnerability caused by hypoxia after TBI. Thus, the authors monitored the extracellular glutamate concentrations following TBI in real time using an enzyme electrode biosensor following TBI, either with or without inducing hypoxia in rats. They also reported that the TBI with moderate hypoxia induced the prolonged efflux of glutamate, which resulted in further cortical damage due to necrosis and apoptosis. These data suggested that neuronal damage after TBI was caused mainly by massive glutamate release that activated

NMDA receptors. Even mild TBI was responsible for glutamate-mediated hippocampal CA3 damage linked to posttraumatic amnesia. In addition, Katoh et al.³⁴ investigated the extracellular concentrations of neuroactive amino acids in the hippocampus by *in vivo* microdialysis. CH injury followed by hypoxia produced a biphasic increase in the concentration of extracellular glutamate and taurine, with an immediate peak after injury and a prolonged plateau after hypoxia. Pre-treatment with dizocilpine maleate (MK-801), a non-competitive NMDA antagonist, did not affect the immediate peak of glutamate after CH injury but significantly diminished the prolonged plateau after hypoxia. These findings suggested that TBI may increase the hypoxic release of glutamate, thereby contributing to increased vulnerability to hypoxia.

The NMDA receptor is clearly involved in the pathophysiology of TBI. A recent study measured the protein and RNA levels of NMDA receptor subunits in the cerebral cortex and hippocampus after injury, thus suggesting that an injury-induced reduction in the expression of the NMDA receptor was one likely mechanism for the impaired experience-dependent neuroplasticity observed in the immature brain following TBI.³⁵ Kumar et al.³⁶ investigated the temporal changes in NMDA receptor subunit (NR1, NR2A, and NR2B) levels in the rat hippocampus after TBI using a Western blot analysis and mRNA expression levels using real-time polymerase chain reaction methods. A Western blot analysis revealed significant decreases in the levels of NR1, NR2A, and NR2B subunit proteins at 6 and 12 hours after injury in the rat hippocampus. The complete recovery of the NR1, NR2A, and NR2B subunit protein levels to that of sham controls was observed at 24 hours after injury. However, analysis did not show any significant change in the mRNA levels at 2, 6, and 12 hours after injury in comparison with sham controls, thus suggesting nontranscriptional change in the levels of these subunits. Thus, TBI can produce transient degradation of NMDA receptor subunits in the hippocampus, which might contribute to temporary memory impairment after injury. As observed in these reports, the hippocampal NMDA receptor subunits, by virtue of their involvement in excitotoxic injury as well as memory association, might play an important role in the pathophysiological mechanisms of TBI.

Calcium-dependent excitotoxic processes and induction of inflammatory cytokines significantly contribute to pathological responses such as apoptotic cell death and glial reactions following TBI. As reviewed by Raghupathi et al.,³⁷ the apoptosis of neurons and glia contributed to the overall pathology of TBI in both humans and animals. While excitatory amino acids, increases in intracellular calcium, and free radicals can all cause cells to undergo apoptosis, *in vitro* studies have determined that neuronal cells can undergo apoptosis via many other pathways. However, the biochemical cascades underlying posttraumatic signal transduction, which causes these pathological alterations are poorly understood. The surge of glutamate might be derived from cortical impact depolarization,³⁸ which immediately induced cytokine gene expression and neurotrophic gene expression within the bilateral cerebral hemisphere.^{39–41}

Recent studies have indicated that TBI induced the expression of neurotrophin-related mRNA and receptors in the rat hippocampus, which triggered downstream mitogen-activated protein kinases (MAPK) cascades through interactions with specific high-affinity tyrosine kinase receptors.^{42–45} The MAPK cascades, which play a crucial role in signal transduction, are activated by phosphorylation in response to a variety of mitogenic signals. The cascades are composed of extracellular signal-regulated protein kinase (ERK), c-Jun NH(2)-terminal kinase (JNK), and p38 pathways. ERK is activated in response to growth factors,⁴⁶ oxidative stress,⁴⁷ and intracellular calcium influx.⁴⁸ ERK plays an important role in the survival, proliferation, and differentiation of various cells. On the other hand, JNK and p38 are activated in response to the presence of inflammatory cytokines, glutamate toxicity, and hyperosmolality. JNK and p38 cause

alterations in transcription factors which lead to neuronal apoptosis.⁴⁹ Otani et al⁵⁰ demonstrated that the immunoreactivity of ERK and JNK significantly increased following TBI in the rat hippocampus. The data presented in that article suggested ERK- and JNK-, but not p38-phosphorylation, to be associated with the molecular sequelae of TBI, and that the discrepancy in the MAPK alterations reflected differences in selective vulnerability between the mechanical and ischemic events in the rat hippocampus. These findings suggested that a distinct MAPK cascade might participate in the selective vulnerability of hippocampal CA3 neurons after TBI. In addition, Raghupathi et al⁵¹ demonstrated the regional activation of JNK and ERK signaling pathways using immunoblotting and immunohistochemistry after TBI. Furthermore, Mori et al⁵² provided the evidence that perturbations in MAPK signal-transduction pathways were involved in the pathophysiology of TBI. Treatment with PD98059, which inhibits the ERK pathway, significantly increased cell survival *in vitro*. ERK pathway inhibition with PD98059 resulted in a significant reduction in the cortical lesion volume 7 days after trauma. The p38 kinase and JNK inhibitor SB203580 had no detectable beneficial effect. These data indicated that critical perturbations in MAPK pathways mediated cerebral damage after acute injury, and that ERK was a novel therapeutic target in traumatic brain injury.

Accumulating data indicate that extracellular proteolysis also plays a critical role in the pathophysiology of neuronal cell death after TBI. The two major systems that modify the extracellular matrix in the brain are the plasminogen activator (PA) and matrix metalloproteinase (MMP) axes. Deleterious effects include the disruption of blood-brain barrier integrity, amplification of inflammatory infiltrates, demyelination, and possible interruption of cell-to-cell and cell-to-matrix interactions that may trigger cell death. In contrast, PA-MMP actions may contribute to the extracellular proteolysis that mediates parenchymal and angiogenic recovery after TBI.⁵³ Asahi et al⁵⁴ showed that the MMP is involved in the pathophysiology of TBI. In particular, MMP-9 knockout mice were protected against TBI. Several authors have so far demonstrated that the resident brain cells secrete MMP after injury, astrocytes are the main source of MMP-9 activity, and the MAPK pathway is activated after mechanical injury, mediating the secretion of MMP-9. These data indicate that the MAPK pathway triggers the upregulation in MMP-9 after trauma, and further suggest that targeting the upstream signaling mechanisms that regulate deleterious MMP-9 activity may reveal new therapeutic opportunities for TBI.^{55,56}

Brain trauma results in neuronal apoptosis and axonal tract damage. These pathologies are worsened by the inflammatory cascade set into motion by the initial injury.⁵⁷ Two pro-inflammatory cytokines released after TBI are tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). Several studies have documented rapid increases in TNF- α and IL-1 β levels after TBI.^{58–60} These pro-inflammatory cytokines stimulated inflammatory cells to release damaging reactive oxygen and nitrogen species, to raise the glutamate levels to excitotoxic levels, to impair the ability of glia cells to buffer extracellular potassium, to compromise the blood-brain barrier, and to attract more inflammatory cells into the brain.^{61,62}

In this article, we reviewed the clinical and experimental evidence for hippocampal damage following TBI. Regardless of the etiology, the symptoms observed contribute to significant disability for many patients. The goal is to maximize recovery and minimize the secondary insults after TBI. Further investigations are needed to better understand hippocampal damage and delineate the best strategies for improving the outcomes after TBI.

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