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Mitogen-Activated Protein Kinase Pathways Following Traumatic Brain Injury

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ABSTRACT

The mechanisms underlying the secondary or delayed cell death in the hippocampus and cerebral hemisphere after traumatic brain injury (*TBI*) *have been poorly understood. Recent data suggesting that TBI may have relationship with both an inflammatory and a neurodegenerative factors are also presented. Mitogen-activated protein kinases* (*MAPK*), *which play a crucial role in signal transduction, are activated by phosphorylation in response to a variety of mitogenic signals. In this article, we review the clinical and experimental evidence for brain damage after TBI. In addition, the MAPK pathways, closely involved in signal transduction after TBI, which could therefore be a new and potentially effective therapeutic target in TBI. Further investigations are therefore necessary to better understand cerebral traumatic damage and delineate the best practice strategies needed to improve the patient outcomes after TBI.*

Keywords: *Traumatic Brain Injury, Mitogen-Activated Protein Kinase, Cell Signaling*

1. Introduction

Posttraumatic amnesia is a common symptom after TBI, and may be related to hippocampal dysfunction [1]. Particularly, memory deficits were found in 90% of patients had made a good recovery after mild and moderate TBI [2]. Patients with mild TBI and persistent postconcussive symptoms have a high incidence of medial temporal lobe injury [3]. 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 [4-7]. However, the pathology of neuronal cell death after TBI and the mechanism of MAPK regulation have not yet to be fully understood. Further investigations will be necessary to elucidate the mechanism of neuronal injury after TBI. We herein review the pathophysiology of TBI and alteration of MAPK after TBI. These findings suggested that a distinct MAPK cascade might participate in the pathophysiological disorder after TBI. In addition, the MAPK cascades could therefore be a new and potentially effective therapeutic target in TBI.

2. Discussion

2.1. Selective Vulnerability in the CA3 Neurons after Experimental TBI

In the review by Lighthall *et al.* [8] regarding experimental TBI models, the authors described and characterized the pathophysiologic changes using a fluid percussion injury (FPI) method and a controlled cortical impact (CI) technique. Chen *et al.* [9] 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 addition, Dixon *et al.* [10] characterized a new FPI model of experimental brain injury to systematically examine the physiologic and histopathologic responses in rats at two levels of injury severity. 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 al.* [11] 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. Several experimental studies have su-

ggested that a selective vulnerability to TBI was observed in hippocampal CA3 neurons [11-13]. Immonen *et al.* [14] 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. The injured neurons were shown as Nissl-stained dark neurons. Ooigawa *et al.* [15] 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. The data suggested that not all Nissl-stained dark neurons inevitably died after TBI. Lowenstein *et al.* [16] showed that neurons of the dentate hilus were vulnerable to a brief, unilateral impact to the extradural surface of the brain using FPI model. This neuronal loss was highly selective since the adjacent dentate granule and pyramidal neurons appeared relatively unaffected. In particular, the mechanism of posttraumatic selective vulnerability of hippocampal CA3 neurons has not yet been fully elucidated.

2.2. Calcium-Dependent Excitotoxic Processes after TBI

Several studies suggested that TBI induced acute neurodegeneration [17] which lead to progressive atrophic changes of the injured cerebral hemisphere [18]. Calcium-dependent excitotoxic processes and induction of inflammatory cytokines significantly contribute to pathologic responses, such as apoptotic programmed cell death [19] and glial reaction following TBI [20]. However, the biochemical cascades underlying posttraumatic signal transduction, which causes these pathological alterations are poorly understood. Matsushita *et al.* [21] suggested that TBI induced neuronal depolarization and excessive excitatory neurotransmitter release, which enhanced glutamate toxicity and led to an increase in intracellular calcium levels [22]. The surge of glutamate might be derived from cortical impact depolarization [23], which immediately induced cytokine genes [24] and neurotrophic genes expression within the bilateral cerebral hemisphere [25, 26]. The NMDA receptor is clearly involved in the pathophysiology of TBI [27,28], 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. Calcium-dependent excitotoxic processes and induction of inflammatory cy- tokines significantly contribute to pathological responses such as apoptotic cell death and glial reactions after TBI. As reviewed by Raghupathi *et al.* [29], 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 [30], which immediately induced cytokine gene expression [31] and neurotrophic gene expression within the bilateral cerebral hemisphere [32,33].

2.3. Mitogen-Activated Protein Kinase Pathways after TBI

Recent studies have indicated that TBI induced the expression of neurotrophin-related mRNA and receptors [34-36] in the rat hippocampus, which triggered downstream mitogen-activated protein kinases (MAPK) cascades through interactions with specific high-affinity tyrosine kinase receptors [37]. The MAPKs are serine/ threonine protein kinases that promote a large diversity of cellular functions in many cell types, 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 (**Figure 1**). ERK is activated in response to growth factors [38], oxidative stress [39], and intracellular calcium influx [40]. Activated ERK can interact with cytoplasmic components or can translocate to the nucleus. Evidence has shown that sustained ERK is translocated to the nucleus [41,42] and nuclear translocated ERK can promote neuronal cell death, regulating transcription [43], which plays an important role in the survival, proliferation, and differentiation of various cells [44]. Recently, a new member of MAPKs, ERK5 has been identified and implicated in neuronal survival [45]. Rapid ERK5 activation was observed in the hippocampal CA3 and dentate gyrus regions after cerebral ischemia [46]. On the other hand, JNK and p38 are activated in response to the presence of inflammatory cytokines [47], glutamate toxicity [48]. JNK and p38 cause alterations in transcription factors which lead to neuronal apoptosis [49]. Several studies suggested the activation of JNK and p38 cascades induced neuronal injury following cerebral ischemia [50,51] and spinal cord injury [52]. Mandell *et al.* [53] demonstrated that the focal mechanical injury induced a rapid activation and spreading of astroglial ERK activation in a defined in vitro model and suggested that the similar mechanism may result in astroglial activation following TBI. However, there has been no reports focusing on the expression and distribution of phosphorylated-MAPKs following TBI in vivo. Otani *et*

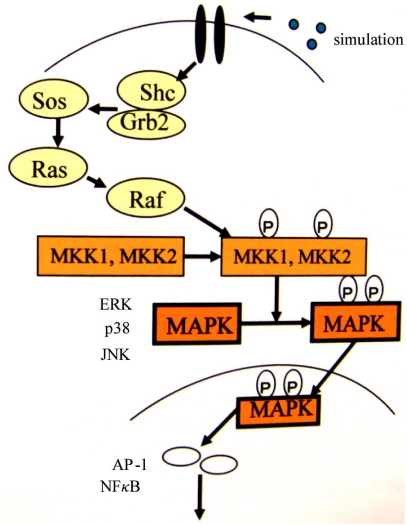

Figure 1. Mitogen-activated protein kinases (MAPKs) play a crucial role in the transduction of signals through protein kinases and protein phosphatases. The MAPK pathways are a ubiquitous group of protein serine and threonine kinases that regulate gene expression through transcription factor activity. The stimulus may transduce to the nucleus to regulate gene expression through a distinct set of MAPK signal transduction cascades, including extracellular signal-regulated kinases 1 and 2 (ERK1, ERK2), p38 mitogen-activated protein (p38), and the c-Jun NH2-terminal kinase (JNK). These pathways are important mediators of the signal transduction responsible for cell growth and proliferation. The nuclear targets of these MAPK signaling pathways are transcriptional factors, such as transcriptional factor activator protein-1 (AP-1) and nuclear factor-kappa B (NFκB), which regulate the expression of various genes.

al. [53] demonstrated that the focal mechanical injury induced a rapid activation and spreading of astroglial ERK activation in a defined in vitro model and suggested that the similar mechanism may result in astroglial activation following TBI. However, there has been no reports focusing on the expression and distribution of phosphorylated-MAPKs following TBI *in vivo*. Otani *et al.* [54] 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 ischemical events in the rat hippocampus. Thus, recent studies have suggested that the activation of JNK and p38 pathways without an activating ERK pathway induced selective CA1 vulnerability to transient forebrain ischemia [55,56]. In addition, the authors investigated, for the first time, the activation of the MAPK pathways in the rat hippocampus following experimental TBI. These findings suggest that a distinct MAPKs cascade might therefore participate in the selective vulnerability of hippocampal CA3 neurons following TBI. Raghupathi *et al.* [57] demonstrated the regional activition of JNK and ERK signaling pathways using immunoblotting and immunohistochemistry following TBI. Most of the pharmacological studies implicating ERK have been carried out using PD98059 or U0126 (which inhibits mitogen-activated protein kinase/ERK kinase, an upstream activator of ERK1). Initially, ERK activation was considered as a promoter of neuronal survival and memory [58]. However, it is now clear that ERK activition can also participate in a variety of neuronal death signals [59]. Mori T *et al.* [60] 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 TBI. Otani N *et al.* [61] studied the effects of inhibition of ERK phosphorylation using MAPK/ERK (MEK) inhibitor U0126 on the histopathological and behavioral outcome after TBI. Thus, the administration of U0126 improved the histopathological and motor functional performance 3, 4, and 5 days after TBI. The authors suggested that the inhibition of the ERK phosphorylation could therefore be a new and potentially effective therapeutic target in TBI. Several studies have shown that U0126 enhanced the regional cerebral blood flow by inducing smooth muscle cells to block the effects of endothelin-mediated vasoconstriction [62]. U0126 has also been shown to reverse the permeability of endothelial cell monolayers increased by vascular endothelial growth factor [63]. In addition, ERK upregulates the extracellular matrix degrading enzyme matrix metalloproteinase-9, which exacerbates the histopathological findings and motor performance after TBI [64]. These results suggest that the neuroprotective effects induced by U0126 may be mediated through a reduction in the vascular permeability thus leading to edema formation after TBI. 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 [65]. Asahi *et al.* [66] 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 [64,67].

2.4. Induction of Inflammatory Cytokines after TBI

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 [68]. Two pro-inflammatory cytokines released after TBI are tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [69,70], which induced astrogliosis [71]. Several studies have documented rapid increases in TNF-α and IL-1β levels after TBI [72-74]. These pro-inflamma- tory 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 [75,76]. Interestingly, recent study showed that over-expression of GFAP induced by TNF-α was significantly attenuated by the ERK inhibitor PD98059. The authors in this article suggested that TNF-α might upregulate GFAP through the ERK pathway [77]. Double immunostaining results in the present study showed that the immunoreactivity for p-ERK was almost exclusively localized in astrocytes surrounding the contusional region after 6 hours of TBI. We speculated that the induction of p-ERK in astrocytes in the late period of TBI has an important role of astroglial reaction led to astrogliosis, which are beneficial for neuronal survival and repairment of damaged blood-brain barrier [78]. Cyclooxygenase-2 (COX-2), a rate-limiting enzyme converting arachidonic acid to prostaglandins and a key player in neuroinflammation, has been implicated in the pathogenesis of TBI, which modulate synaptic transmission and plasticity and cause neurodegeneration after TBI. The actions of these COX-2 metabolites are likely mediated by MAPK and inositol 1,4,5-trisphosphate (IP3) signal transduction pathways. In addition, recent work [79,80] shows that PGE2-G-enhanced hippocampal GABAergic and glutamatergic synaptic transmissions are not mediated via PKA and PKC pathways, but appear to be mediated through ERK, p38, IP3, and NF-κB signal transduction pathways. Yang *et al.* [81] demonstrated that the PGE2-G-induced increase in hippocampal LTP is attenuated by an IP3 inhibitor, indicating the involvement of the IP3-mediated mobilization of intracellular Ca^{2+} in PGE2-G-induced increase in LTP. The involvement of ERK and p38 pathways is further supported from the molecular evidence. PGE2-G induces a time-dependent phosphorylation of ERK and p38MAPK, and this phosphorylation is attenuated by ERK and p38 inhibitors. TBI leads to the development of gliosis, but little is known about the signal transduction mechanisms that underlie this process. Gliosis is characterized by hypertrophic and hyperplastic changes of astrocytes in response to brain injury. ERK was widely expressed in adult brain with high levels apparent in neocortical neuronal cell bodies and dendrites [82]. Johanson *et al.* [83] suggested a retrograde axonal transport of p-ERK might play a role in neurotrophic signal transmission from the nerve terminal to the cell body in the rat sciatic nerve. The retrograde axonal transport of p-ERK is of limited value because it may take much time to reach the soma of neurons [84]. An induction of p-ERK was observed in astrocytes surrounding pyramidal CA3 neurons and contusional area at 6 hours after TBI [54,85], which might be derived from intracellular signal transduction in response to TBI. Mandell *et al.* [86] assessed that ERK phosphorylation triggered an astroglial reaction which led to reactive astrogliosis, which has both beneficial and detrimental consequences for the functional recovery of neurons. A recent study indicated that reactive astrocytes have a beneficial effect on both neuronal survival and the repair of the damaged blood-brain barrier [87]. The prolonged phosphorylation of p-ERK in astrocytes might thus play a crucial role in the promotion of cell survival in the late period of TBI. Reactive astrogliosis is the most prominent response to diverse forms of TBI. TBI induced GFAP gene expression, which might be a sensitive molecular marker for evaluating the global response in progressive glial scarring in the rat brain [88]. On the other hand, several reports showed that there were close relationships between inflammation, cytokine production, and astrogliosis [89]. Reactive astrocytes induced the expression of a variety of molecules such as neurotrophin and growth factor families [90]. Mandell *et al.* [53] investigated the mechanism of ERK activation with the primary cultured astroglial monolayers subjected to focal mechanical injury and demonstrated that cortical focal lesion induced a rapid spreading of astroglial ERK activation.

3. Conclusions

We herein review the pathophysiology of TBI and alteration of MAPK after TBI. These findings suggested that a distinct MAPK cascade might participate in the pathophysiological disorder after TBI. In addition, the MAPK cascades could therefore be a new and potentially effective therapeutic target in TBI. However, the pathology of neuronal cell death after TBI and the mechanism of MAPK regulation has not yet to be fully understood. Further investigations will be necessary to elucidate the effect of MAPK pathway after TBI.

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