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Review

Molecular mechanisms in the pathogenesis of traumatic brain injury

S.K. Ray[1], C.E. Dixon[2] and N.L. Banik[1]

[1]Department of Neurology, Clinical Science Building, Medical University of South Carolina, Charleston, SC, USA and

[2]Department of Neurosurgery, Brain Trauma Research Center, University of Pittsburgh, Pittsburgh, PA, USA

Summary. Traumatic brain injury (TBI) is a serious neurodisorder commonly caused by car accidents, sports related events or violence. Preventive measures are highly recommended to reduce the risk and number of TBI cases. The primary injury to the brain initiates a secondary injury process that spreads via multiple molecular mechanisms in the pathogenesis of TBI. The events leading to both neurodegeneration and functional recovery after TBI are generalized into four categories: (i) primary injury that disrupts brain tissues; (ii) secondary injury that causes pathophysiology in the brain; (iii) inflammatory response that adds to neurodegeneration; and (iv) repair-regeneration that may contribute to neuronal repair and regeneration to some extent following TBI. Destructive multiple mediators of the secondary injury process ultimately dominate over a few intrinsic protective measures, leading to activation of cysteine proteases such as calpain and caspase-3 that cleave key cellular substrates and cause cell death. Experimental studies in rodent models of TBI suggest that treatment with calpain inhibitors (e.g., AK295, SJA6017) and neurotrophic factors (e.g., NGF, BDNF) can prevent neuronal death and dysfunction in TBI. Currently, there is still no precise therapeutic strategy for the prevention of pathogenesis and neurodegeneration following TBI in humans. The search continues to explore new therapeutic targets and development of promising drugs for the treatment of TBI.

Key words: TBI, Secondary injury, Calpain, Neurodegeneration, Calpain inhibitors

Introduction

It is noticed that TBI is often associated with an elevation of ventricular intracranial pressure (ICP). A high ICP indicates poor outcome after TBI (Marshall et al., 1979). Steroids were known to improve neurological function temporarily by diminishing brain swelling associated with brain tumors. Because brain swelling also occurs in TBI, steroids were thought to be effective for the treatment of TBI. But, a series of clinical trials made it clear that steroids did neither significantly reduce ICP nor improve neurological function of the patients with severe TBI. Indeed, the pathophysiology of TBI is totally different from that of brain tumors. It is the "secondary injury" that significantly contributes to the prognosis of patients suffering from TBI. The development of "fluid-percussion model" of mechanical brain injury (Sullivan et al., 1976) was able to demonstrate diffuse axonal injury (DAI). This model explained that high-speed motor vehicle accidents could cause DAI without an impact to the head. Later, the development of "controlled cortical contusion model" of experimental brain injury (Lighthall, 1988) was successful to show the occurrence of DAI as well as cortical contusion, a very common component of severe TBI in humans. With the use of the rodent controlled cortical contusion model (Dixon et al., 1991), researchers were able to identify the pivotal role of oxygen free radicals (Kontos, 1989; Hall, 1993) in the progression of secondary injury. However, a clinical trial with the scavenger of oxygen free radicals (Muizelaar et al., 1993) alone did not result in significant functional recovery after severe TBI. Recent studies have documented the involvement of other factors such as glutamate (Bullock and Fujisawa, 1992) and Ca^{2+}-dependent cysteine protease calpain (Kampfl et al., 1996; Saatman et al., 1996a; Posmantur et al., 1997), which are crucial for the pathogenesis during secondary injury. Several drugs are being tested to prevent the harmful effects of these crucial factors during secondary injury (Saatman et al., 1996b; McIntosh et al., 1998; Kupina et al., 2001). Current strategy for the treatment of TBI should be designed to control secondary injury before it causes an elevation of ICP and neurodegeneration.

Offprint requests to: Naren L. Banik, Ph.D., Department of Neurology, Clinical Science Building, Medical University of South Carolina, 96 Jonathan Lucas Street, Suite 309, Charleston, SC 29425, USA. Fax: 843-792-8626. e-mail: baniknl@musc.edu

Epidemiology of TBI

A study in the United States alone indicates that an estimated 200 thousand victims of severe TBI require

admission to the hospital every year and most of them become permanently disabled, and another 1.74 million victims experience mild TBI requiring doctor visit or temporary disability of one day at least (Waxweiler et al., 1995). Total costs for medical care and rehabilitation of patients with TBI are estimated to exceed $40 billion annually. Typically young people between the ages of 15 and 30 years are at the greatest risk of suffering from TBI. Men are approximately twice as likely as women to go through a TBI. Approximately 52 thousand deaths in the United States are attributed to TBI every year. Common causes of fatal TBI are automobiles, firearms, bicycles, falls, sports and recreational activities, and risky jobs. Automobile accidents are responsible for the largest percentage of all types of TBI, and also the predominate cause of fatal TBI in whites. Gunshot wounds to the head account for the highest number of deaths in young black males, followed by Hispanic and Native American males. Some forms of TBI can be prevented. The use of automobile seat belts may reduce the risk of TBI-related morbidity and mortality. The habit of wearing helmets may help decrease the number and severity of TBI in motorcyclists and bicyclists. Other preventive measures such as speed limit and drunk driving laws may also help reduce the number of TBI cases. It is warranted to focus on the prevention of a large proportion of deaths from TBI by limiting access to firearms, particularly handguns.

Types of TBI

An epidemiological study in the United States classified the TBI severity (Frankowski, 1986) that combined the symptoms and extent of brain injury as previously determined by the Glasgow Coma Scale (GCS) score (Teasdale and Jennett, 1974). As the most widely used classification, the GCS enables quantification of TBI severity based on patient's response to commands and stimuli. The GCS scores of 3 to 8, 9 to 12, and 13 to 15 represent severe, moderate, and mild TBI, respectively. TBI severity can also be classified into main five categories based on locations: (i) extracranial injury, (ii) skull fracture, (iii) focal injury, (iv) diffuse injury, and (v) penetrating injury. Diagnostic procedures and practice patterns may influence TBI classification and hospitalization. A TBI may be considered severe in terms of its cost to the society, even if the TBI patient is not hospitalized.

Pathophysiology of TBI

The events leading to pathophysiology as well as functional recovery after TBI fall into four categories: (i) primary injury that disrupts brain tissues at the moment of mechanical impact; (ii) secondary injury that causes pathophysiology in the brain with the production of high levels of lactate, oxygen free radicals, interleukins, glutamate, and intracellular free Ca^{2+} in response to primary injury; (iii) inflammatory response that adds to neurodegeneration with the help of oxygen free radicals and toxic neurochemicals; and (iv) repair-regeneration that remains a poorly understood process, and it may contribute to neuronal regeneration, axonal repair and partial neurological recovery following TBI.

Primary injury

Generally, TBI is triggered by an external mechanical impact to the head. An impact load causes TBI through a combination of two injury mechanisms such as contact and inertial forces (Graham et al., 1995). Contact forces prevent the head from moving after the impact. Inertial forces set the head in acceleration (translational or rotational or both) with and without a contact force. The two main patterns of head trauma are focal and diffuse injuries. Contact forces cause focal injuries such as skull fracture, epidural hematoma, coup contusion, and subdural hematoma. Inertial forces with only pure translational acceleration cause focal injuries such as contracoup contusion, intracerebral hematoma, and subdural hematoma. The inertial forces cause diffuse injuries. The most common form of inertial forces is the angular acceleration, a combination of translational acceleration and rotational acceleration, which produces every type of head trauma except skull fracture and epidural hematoma. Rotational acceleration as a significant component of the injury mechanism produces concussion and DAI.

Secondary injury

It is developed over a period of hours or days after the initial impact to the head. Secondary injury is associated with synthesis and release of neurochemicals that alter cerebral blood flow, ion homeostasis, and metabolism. Most of the post-traumatic neurochemical mediators of secondary injury may act as neurodestructive compounds. Identification of those neurodestructive compounds and time of their pathological actions can help design therapeutic strategies to attenuate neuronal damage following TBI.

Oxygen free radicals and lipid peroxidation

Post-traumatic ischemia activates a cascade of metabolic events leading to generation of oxygen free radicals (Kontos and Povlishock, 1986; Ikeda and Long, 1990; Traystman et al., 1991). Post-traumatic non-ischemic event such as the increase of intracellular free Ca^{2+} concentration (through receptor-gated or voltage-dependent ion channels) may also induce release of oxygen free radicals from mitochondria (Kontos and Povlishock, 1986; Tymianski and Tator, 1996). Stimulation of enzymatic activities of cyclooxygenase, monoamine oxidase, and nitric oxide synthase can produce oxygen free radicals. The highly reactive oxygen free radicals can cause damage by lipid peroxidation in cell membrane, and oxidation of

intracellular proteins and nucleic acids. TBI may activate phospholipases A2 and C to hydrolyze membrane phospholipids releasing arachidonic acid. Formation of pathogenic compounds such as free fatty acids (Dhillon et al., 1994, 1995), leukotrienes (Kiwak et al., 1985; Dhillon et al., 1996), and thromboxane B2 (DeWitt et al., 1988) from the arachidonic acid cascade have been associated with neurodegeneration and poor outcome after experimental TBI.

The inhibitors of arachidonic acid cascade are partially effective in experimental CNS injury. Ibuprofen and indomethacin, inhibitors of cyclooxygenase, are effective in improving cerebral metabolism in rats with cortical freeze injury (Pappius and Wolfe, 1983), and reducing neurological dysfunction in mice with weight-drop brain injury (Hall, 1985). Extracellular ascorbate was increased in the cerebral cortex after weight-drop brain injury in rats (Hillered et al., 1990), indicating a deficiency in intracellular anti-oxidant defense against oxygen free radicals and lipid peroxidation. Anti-oxidants and free radical scavengers have been beneficial in experimental models of brain trauma (Wei et al., 1981; Stein et al., 1991; Marklund et al., 2001). For example, administration of 21-aminosteroid U74006F, a potent oxygen free radical scavenger, could reduce cerebral edema and mortality (McInctosh et al., 1992), and improve motor function (Sanada et al., 1993) of rats with brain injury. Subsequently, other experimental brain injury studies also suggested 21-aminosteroids to be effective in treating axonal injury (Marion and White, 1996) and in reducing microvascular permeability at the site of cortical injury (Mathew et al., 1996). Although 21-aminosteroids generated promising results in various animal models of TBI, they were not significantly effective in the treatment of severe TBI in humans.

Pre-treatment and acute post-treatment with D, α-tocopheryl succinate plus polyethylene glycol attenuated motor deficits following TBI (Clifton et al., 1989). Analogues of α-tocopherol have also been reported to be neuroprotective in mice following TBI (Grisar et al., 1995). Lidocaine, a local anesthetic, has been found to be a potent scavenger of hydroxyl radicals (Das and Misra, 1992). Administration of lidocaine has been reported to attenuate post injury neurological and motor function, but not cognitive function (Muir et al., 1995). Deferoxamine is an iron-chelating agent that can inhibit the iron-dependent hydroxyl radical production, and has been reported to improve spatial memory performance following TBI (Long et al., 1996). Interestingly, deferoxamine was found not to improve functional outcome when combined with moderate hypothermia treatment (Heegaard et al., 1997). Superoxide dismutase (SOD) is a metalloenzyme, which catalyses the dismutation of superoxide ion into oxygen and hydrogen peroxide. Administration of polyethylene glycol-conjugated SOD has been reported to reduce motor, but not Morris water maze deficits following TBI (Hamm et al., 1996). OPC-14117 is a superoxide scavenger that has been reported to attenuate tissue damage (Mori et al., 1998) and behavioral deficits (Kawamata et al., 1997) following TBI. Early treatment with LY341122, an inhibitor of lipid peroxidation and an antioxidant, has been reported to provide significant histopathological protection (Wada et al., 1999). Penicillamine is a scavenging compound that has been reported to improve motor performance in mice after TBI (Hall et al., 1999). The pineal hormone melatonin, a scavenger of free radicals, has been found to reduce contusion volume following cortical impact in rats (Sarrafzadeh et al., 2000). Endothelin-1, a 21-amino acid peptide, has been closely linked to oxidative stress after traumatic brain injury (Sato and Noble, 1998). The endothelin receptor sub-type A antagonist, Ro 61-1790 has been shown to attenuate Purkinje cell loss in the cerebellum following TBI.

Sequestration of calcium loads within the mitochondrial matrix can open the mitochondrial permeability transition pore leading to cellular oxidative and metabolic stress. Acute treatment with cyclosporin A (CsA), an inhibitor of Ca^{2+}-induced mitochondrial permeability transition pore, has been reported to reduce tissue damage (Okonkwo et al., 1999; Scheff and Sullivan, 1999; Sullivan et al., 2000b) and improve motor function (Riess et al., 2001) following TBI in rats. A single dose of CsA has also been reported to blunt axonal damage following TBI (Buki et al., 1999). Treatment with creatine, a common food supplement, reduced cortical damage in both mice and rats (Sullivan et al., 2000a). Protection seems to be related to creatine-induced maintenance of mitochondrial bioenergetics. Mitochondrial membrane potential was significantly increased, intramitochondrial levels of reactive oxygen species and calcium were significantly decreased, and adenosine triphosphate levels were maintained. Induction of mitochondrial permeability transition was significantly inhibited in animals fed creatine. This food supplement may provide clues to the mechanisms responsible for neuronal loss after TBI and may find it useful as a neuroprotective agent against acute and delayed neurodegenerative processes.

Excitatory amino acids and excitotoxicity

Glutamate and aspartate are excitatory amino acids (EAA) that are released in high concentrations in extracellular space and cerebrospinal fluid (CSF) soon after TBI (Faden et al., 1989; Palmer et al., 1993). Two sequential mechanisms have been proposed for EAA induced cell death or excitotoxicity that includes (i) an influx of chloride and sodium ions (Cl^- and Na^+) leading to acute neuronal and glial swelling, and (ii) an influx of calcium ion (Ca^{2+}) leading to delayed damage (Choi, 1987; Choi et al., 1987). Although glutamate binds to all EAA receptors, selective ligands have been used to characterize three major types of EAA receptors. The first type of glutamate receptor binds N-methyl-D-aspartate (NMDA) and is known as NMDA receptor,

which is a membrane complex associated with a monovalent and divalent ion channel (ionophore). Activation of NMDA receptor with subsequent opening of the ionophore in a voltage-dependent manner allows the influx of Na^+ and Ca^{2+} into the cell (Mayer et al., 1984). Opening of the ionophore is facilitated by binding of glycine to a specific site on the NMDA receptor (Kleckner and Dingledine, 1988). The activity of NMDA receptor can also be modulated by binding of polyamines, which may augment or inhibit receptor activation (Ransom and Stec, 1988). The zinc ion (Zn^{2+}) is also known to antagonize the binding of glycine to its site (Yeh et al., 1990). The second type of glutamate receptor binds excitatory ligand α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainic acid (AMPA/KA) and is referred to as AMPA/KA receptor (or non-NMDA receptor), which is associated with a monovalent ion channel. The activation of AMPA/KA receptor opens the associated ionophore in a non-voltage-dependent manner, allowing the influx of Na^+ and efflux of K^+ from the cell (Reynolds and Miller, 1988). A subtype of AMPA/KA receptor may also be permeable to Ca^{2+} (Iino et al., 1990). The third type of glutamate receptor is metabotropic receptor that, unlike ionotropic receptors, is associated with the activation of an intracellular second messenger. Binding of glutamate to this type of receptor activates phospholipase C (Wei et al., 1982), which may induce the synthesis of inositol triphosphate for releasing Ca^{2+} from the intracellular stores (Sugiyama et al., 1987). The regional distribution of NMDA and non-NMDA receptors is directly related to excitotoxicity in specific regions of the brain following TBI (Miller et al., 1990). Hippocampus, which plays a prominent role in learning and memory, has a high density of glutamate receptors (Monaghan and Cotman, 1986). Hippocampal dysfunctions, including a suppression of long-term potentiation (Miyazaki et al., 1992) and deficiency in learning and memory (Smith et al., 1993a), have been reported following TBI in rats.

Pre-treatment with competitive NMDA receptor antagonists has been effective to reduce EAA release following TBI (Panter and Faden, 1992). However, a multi-center human trial of Selfotel (a competitive NMDA receptor antagonist) in the United States and Europe was prematurely terminated because of serious side effects associated with competitive NMDA receptor antagonism. Pretreatment with noncompetitive NMDA receptor antagonist such as phencyclidine (Hayes et al., 1988) or MK-801 (McIntosh et al., 1988) attenuated neurological dysfunctions following TBI in rats. Recent studies also reported that pretreatment with MK-801 could reduce extracellular EAA rise in hippocampus (Katoh et al., 1997), and enhance recovery of spatial memory (Phillips et al., 1997) in rats after TBI. Administration of ketamine, blocker of NMDA receptor-associated ion channel, improved cognitive functions after fluid-percussion brain injury (Smith et al., 1993b) and reduced expression of several immediate early genes (c-fos, fos B, jun B, and jun D) in cerebral cortex and hippocampal dentate gyrus after focal mechanical brain injury (Belluardo et al., 1995). Kynurenate (KYNA), an antagonist of NMDA receptor-associated glycine binding site, provided neuroprotection by preventing loss of hippocampal neurons following TBI in rats (Hicks et al., 1994). Administration of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), which has higher affinity for AMPA/KA receptors than the glycine-binding site of NMDA receptor, could reduce metabolic dysfunction following brain injury (Kawamata et al., 1992).

Dynorphin and neurodegeneration

Regional increase in endogenous opioid dynorphin, a 17-amino acid peptide, has been found to correlate with regional neurodegeneration after experimental brain injury (McIntosh et al., 1987a). Dynorphin is thought to be an endogenous ligand for the κ-opioid receptor (Yoshimura et al., 1982), and has previously been implicated as a mediator of secondary injury in spinal cord trauma (Faden et al., 1985). Microinjections of dynorphin and other κ-opioid agonists exacerbate neurodysfunction in rats with brain injury (McIntosh et al., 1994), supporting the concept that endogenous opioid peptides contribute to the pathophysiology of TBI. Impairment of the tail-flick reflex or motor function due to dynorphin administration could be prevented by treatment with NMDA antagonists (Caudle and Isaac, 1988; Bakshi and Faden, 1990a). Also, administration of antagonists of NMDA receptor-associated glycine binding site could limit dynorphin-induced neurological dysfunctions (Bakshi and Faden, 1990b). These studies suggest that dynorphin-induced neurological dysfunctions involve the release of EAA and excitotoxicity. However, the role of opioid receptors in dynorphin-mediated neurodegeneration has been controversial. Some studies have shown attenuation of dynorphin-induced paralysis with opioid receptor antagonists (Przewlocki et al., 1983; Spampinato and Candeletti, 1985), whereas other studies have not found prevention of paralysis with κ-opioid antagonists (Stevens and Yaksh, 1986; Long et al., 1989). A later report provides experimental evidence that both opioid and non-opioid mechanisms may play a role in dynorphin-induced neurological dysfunctions (Faden, 1990). Subsequent studies supported this hypothesis. For example, dynorphin administration impaired motor function through an opioid mechanism (Bakshi et al., 1990), and caused neurodegeneratiion through a non-opioid mechanism (Faden, 1992). Accumulation of endogenous dynorphin (McIntosh et al., 1987b) as well as release of EAA (Faden et al., 1989) as secondary injury factors following TBI may be limited by treatment with dynorphin antiserum or κ-opioid antagonists (Faden, 1990) and NMDA antagonists (Faden and Simon, 1988).

Acetylcholine and cognitive deficits

Increase in concentration of acetylcholine and

decrease in binding of cholinergic receptors in the brain have been found in experimental brain trauma (West et al., 1981). Cholinergic hyper function occurred at acute phase of trauma, but it changed to cholinergic hypo function at the chronic phase after injury (Saija et al., 1988; Dixon et al., 1996). Increased activity of cholinergic systems or alteration of cholinergic receptors in specific brain regions after TBI may contribute to neurobehavioral dysfunction (Lyeth and Hayes, 1992). Activation of cholinergic system after microinjection of carbachol, a cholinergic agonist, manifests a reversible loss of consciousness identical to that occurs after fluid-percussion brain injury (Leonard et al., 1994). Both depletion of acetylcholine concentrations and obstruction of muscarinic cholinergic receptors in the brain substantially attenuated the transient loss of consciousness and enduring neurodysfunctions associated with fluid-percussion brain injury (Hayes et al., 1984). Administration of scopolamine, an anti-cholinergic compound, decreased neurologic deficits in experimental brain injury (Lyeth et al., 1992; Phillips et al., 1997), indicating involvement of cholinergic system in cognitive dysfunction. To this end, post-injury administration of BIBN 99, a selective antagonist of the muscarinic M2 cholinergic receptor (Pike and Hamm, 1995), or chronic administration of LU 25-109-T, a partial muscarinic M1 cholinergic receptor agonist (Pike and Hamm, 1997), has been shown to improve cognitive function following experimental brain trauma in rats. Probably, a change from cholinergic hyper function to hypo function during the pathophysiological process of injury requires a change in therapeutic strategy from cholinergic antagonists to agonists. Taken together, the experimental results suggest that anti-cholinergic agents may restore reflexive and motor function in acute post-traumatic period, while cholinomimetic compounds may reduce long-term cognitive dysfunction. Since these therapeutic agents work in the opposite directions with respect to their pharmacological effects, the timing of therapy with these agents appears to be critical.

Altered ion concentrations and pathogenesis

Potassium ion (K^+) release into the extracellular space has been detected after TBI (Takahashi et al., 1981). Such K^+ release seems to be related to widespread depolarization (Takahashi et al., 1981; Katayama et al., 1990), and spreading of depression in cerebral cortex (Sugaya et al., 1975). Acute increases in K^+ interfere with membrane transport systems, metabolisms, and synaptic functions (Kimelberg et al., 1979). High levels of extracellular K^+ may also disrupt energy homeostasis after brain injury (Hansen, 1985). Further K^+ stimulates oxygen uptake in glial cells (Hertz et al., 1973) and deprives traumatized neurons of their oxygen supply. As a result anoxic neuronal damage may occur in brain regions after the injury (Siesjo, 1981).

Magnesium ion (Mg^{2+}) is critical for such cellular processes as glycolysis, respiration, oxidative phosphorylation, the biosyntheses of DNA, RNA and protein, and maintenance of Na^+ and K^+ gradients (Aikawa, 1980). A significant decrease in intracellular free Mg^{2+} concentrations soon after TBI has been reported (Vink et al., 1988). Additional investigation indicated prolongation of the decline in Mg^{2+} concentrations in the brain after TBI in rats (Vink et al., 1996). Decreased Mg^{2+} concentrations may impair glucose utilization, energy metabolism, oxidative phosphorylation, and biosynthetic pathways, contributing to regional neurodegeneration after brain trauma. As Mg^{2+} regulates transport and accumulation of calcium ion (Ca^{2+}) in the cells, an alteration in Mg^{2+} concentrations in the brain may cause Ca^{2+} mediated neurotoxicity after TBI. Exogenous magnesium has been demonstrated to be neuroprotective to both functional and morphological deficits following TBI. Administration of magnesium 1-hour post-injury produced significant improvement in neurological function at 18 and 48 hours after injury (Feldman et al., 1996). Animals treated with $MgCl_2$ for 30 min after injury has been reported to protect against neurological deficits (McIntosh et al., 1989). One-hour post-treatment with $MgCl_2$ has been reported to reduce TBI-induced damage to the cortex, but did not alter post-traumatic cell loss in the CA3 region of the ipsilateral cortex (Bareyre et al., 2000). Administration of $MgSO_4$ has been reported to attenuate recovery of function when administered up to 12 hours post injury (Heath and Vink, 1999).

Ca^{2+} plays an important role in initiation of the pathophysiological pathways leading to neuro-degeneration after CNS trauma (Tymianski and Tator, 1996; McIntosh et al., 1997). The elevated intracellular Ca^{2+} levels have been reported in brain regions after TBI (Shapira et al., 1989; Fineman et al., 1993). The decrease in extracellular Ca^{2+} levels following cortical compression contusion brain injury in rats was associated with profound functional disabilities (Nilsson et al., 1993), and the pre-treatment with glutamate receptor antagonists did not decrease in extracellular Ca^{2+} levels (Nilsson et al., 1996). A recent study suggested that excessive intracellular Ca^{2+} resulting from TBI in rats was adsorbed on mitochondrial membrane to inhibit electron transport chain and energy metabolism (Xiong et al., 1997).

It has been proposed that activated ion channels following TBI may contribute to prolonged changes in Ca^{2+} homeostasis (Gennarelli et al., 1998). Ca^{2+} channel blockers to reduce excessive accumulation of intracellular Ca^{2+} have been examined as possible neuroprotective agents for experimental TBI. Post injury administration of the voltage-sensitive Ca^{2+} channel blocker Ziconotide (also SNX-111 and CI-1009) has been reported to attenuate motor and cognitive deficits (Berman et al., 2000). Similarly, post-injury treatment with LOE 908, a broad-spectrum inhibitor of voltage-operated cation channels and store-operated cation channels has been demonstrated to reduce neuromotor

and visuospatial memory deficits (Cheney et al., 2000). Pharmacologically blocking Ca^{2+} entry has been another important strategy to reduce post-injury excitotoxicity. Treatment with (S)-emopamil, a Ca^{2+} channel blocker, has been reported to attenuate post-injury motor deficits (Okiyama et al., 1992). Inhibiting polyamine-dependent Ca^{2+} influx is another therapeutic target for attenuating post-injury excitotoxicity. Ifenprodil, a polyamine-site NMDA receptor antagonist, has been reported to reduce cortical injury volume after TBI (Dempsey et al., 2000).

Administration of voltage-sensitive Ca^{2+} channel blockers provided little success in treating brain injury in humans (Baethman and Jansen, 1986; Robinson and Teasdale, 1990). Dihydropyridine Ca^{2+} channel blockers including nimodipine have recently been evaluated in clinical trials in TBI. Early trials reported beneficial effects of nimodipine in severe head injury in humans (Kostron et al., 1984). More recent trials with both nicardipine and nimodipine did not show clinical benefit in patients with TBI (Compton et al., 1990; Teasdale et al., 1992). Thus, therapeutic efficacy of Ca^{2+} channel blockers in TBI remains controversial. Experimental brain injury in rodent models indicated the increase in mRNA expression of immediate early genes (IEG) such as c-fos (Phillips and Belardo, 1992), and c-jun and jun B (Raghupathi and McIntosh, 1996). The pattern of induction of IEG in fluid-percussion brain injury is similar to that found in seizure (Gass et al., 1993) and ischemia (Wessel et al., 1991; An et al., 1993). However, it is not yet confirmed that induction of IEG contributes to the pathophysiological process in TBI. Members of the fos and jun families function as transcription factors and may mediate adaptive responses in the stimulated nervous system (Morgan and Curran, 1991). The heterodimer of c-Fos and c-Jun proteins regulates the expression of genes for endogenous opioid peptide (Morgan and Curran, 1991), amyloid ß-protein precursor (Quitschke and Goldgaber, 1992), and nerve growth factor (D'Mello and Heinrich, 1991), all of which are overexpressed in TBI (McIntosh et al., 1987a; Pierce et al., 1996). Further, the expression of c-fos (Smeyne et al., 1993) and c-jun (Dragunow et al., 1993) has been associated with apoptosis or programmed cell death (PCD), which is also known to occur in TBI (Colicos and Dash, 1996; Yakovlev et al., 1997; Conti et al., 1998; Newcomb et al., 1999).

Activation of cysteine proteases and apoptosis

Cysteine proteases such as caspase and calpain can play important role for mediation of cell death following TBI. Although activation of caspase-3 via the extrinsic and intrinsic apoptotic pathways after moderate TBI has been documented (Beer et al., 2000; Keane et al., 2001), experimental studies strongly suggest that calpain is more important than caspase-3 for mediation of cell death after TBI (Kampfl et al., 1997; Pike et al., 1998, 2001; Hayes et al., 1999). Calpain activation may occur upstream of caspase-3 for mediation of apoptosis as suggested by recent in vitro (Waterhouse et al., 1998) and in vivo (Ray et al., 2001) studies. The increase in intracellular Ca^{2+} after TBI certainly activates Ca^{2+}-dependent proteases including calpain, which mediates cytoskeletal protein degradation and neurodegeneration in humans (McCracken et al., 1999; Huang and Wang, 2001) and rodents (Kampfl et al., 1996, 1997; Saatman et al., 1996a; Hayes et al., 1999; Pike et al., 2001). Overexpression and activation of calpain degrade neurofilament protein (Banik et al., 1997) and α-fodrin (Ray et al., 1999a) following spinal cord injury in rats. Thus, a pathophysiological role for calpain has also been implicated in spinal cord trauma (Banik et al., 1999). Recent studies used calpain inhibitors such as calpeptin and MDL-28170 (Fig. 1) to demonstrate the involvement of calpain in apoptotic death of rat glial (Ray et al., 1999b) and neuronal (Ray et al., 2000) cells, respectively. Other calpain inhibitors such as calpain inhibitor II, AK295, and SJA6017 (Fig. 1) in rodent models attenuated the loss of cytoskeletal proteins after cortical impact brain injury (Posmantur et al., 1997) and improved functional outcome after fluid-percussion

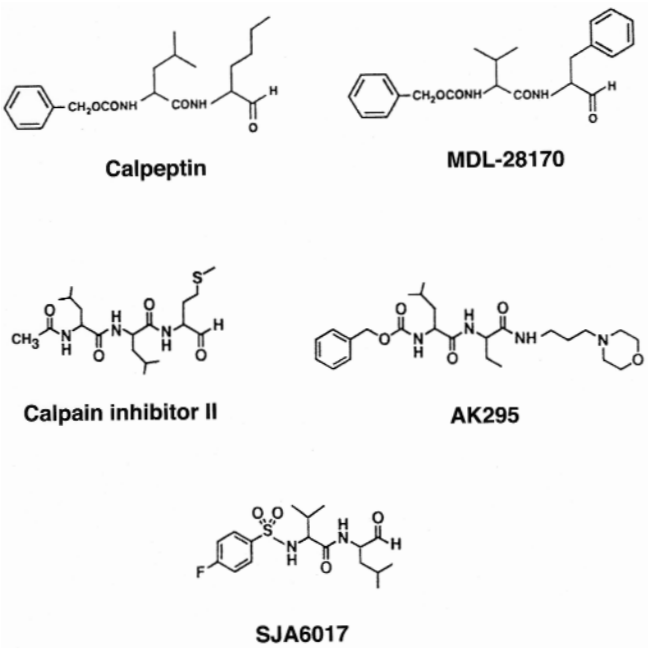

Fig. 1. Various calpain inhibitors known to inhibit CNS cell death. These are cell permeable and active site-targeted calpain inhibitors. Calpeptin and MDL-28170 are structurally similar synthetic N-protected dipeptidyl aldehyde. Calpeptin and MDL-27180 inhibited apoptosis of C6 glial (Ray et al., 1999b) and PC12 neuronal (Ray et al., 2000) cells in culture. Calpain inhibitor II is a synthetic tripeptidyl aldehyde capable of preventing proteolysis and neuronal apoptosis (Villa et al., 1998). AK295 is a synthetic dipeptidyl α-keto amide that inhibits calpain-mediated neurodegeneration in vivo (Bartus et al., 1994). SJA6017 is a new N-protected dipeptidyl aldehyde capable of inhibiting calpain (Fukiage et al., 1997). Neuroprotection in rodent models of TBI has been reported using calpain inhibitor II (Posmantur et al., 1997), AK295 (Saatman et al., 1996b), and SJA6017 (Kupina et al., 2001).

brain injury (Saatman et al., 1996b) and diffuse brain injury (Kupina et al., 2001). Calpain inhibitors are capable of providing neuroprotection both in vitro and in vivo models suggesting that calpain inhibition can be an important therapeutic strategy in TBI.

Inflammatory response

TBI can induce neuronal cells to synthesize and secret inflammatory cytokines such as the peptides of interleukin (IL) family and tumor necrosis factor-α (TNF-α). The patients with severe head injuries had increased levels of IL-1, IL-6, and TNF-α in circulation and cerebro spinal fluid (Young et al., 1988; Goodman et al., 1990; McClain et al., 1991; Ott et al., 1994). Experimental TBI in animal models showed upregulation of IL-1, IL-6, and TNF-α (Woodroofe et al., 1991; Taupin et al., 1993; Shohami et al., 1994). The light and electron microscopic studies indicated a rapid microglial reaction in the dentate gyrus following induction of lesion in the rat cortex (Gehrmann et al., 1991). Activated microglia may be responsible for production of IL-1 and IL-6 in rat brain following mechanical injury (Woodroofe et al., 1991). The accumulation of activated microglia has temporally been associated with vulnerability of Purkinje cells to TBI (Fukuda et al., 1996). Experimental brain injury in rats induced mRNA expression of both IL-1ß (Fan et al., 1995) and TNF-α (Fan et al., 1996) within the brain regions. The induction of mRNA expression of these cytokines occurred concomitantly with an increase in mRNA expression of glial fibrillary acidic protein (GFAP), an indication of astrogliosis in the pathophysiological process of TBI. Astrogliosis has later been shown to be associated with the upregulation of inflammatory cytokines such as IL-1α, IL-1ß, and TNF-α in traumatic murine brain (Rostworowski et al., 1997). Percussive injury to human cerebral microvascular endothelium in culture induced production of IL-1ß and TNF-α (Gourin and Shackford, 1997), suggesting participation of endothelial cells in the inflammatory response after TBI. Acute increase in IL-1ß and TNF-α may trigger synthesis and release of highly neurotoxic agents such as arachidonic acid and its metabolites (Rothwell and Relton, 1993).

Breakdown of blood-brain-barrier (BBB) and infiltration of peripheral immune cells (neutrophils and macrophages) into the brain parenchyma have been reported previously in fluid-percussion brain injury in rats (Cortez et al., 1989) and recently in human brain contusion (Holmin et al., 1998). Infiltration of NK cells, helper T cells, and cytotoxic T cells also occurred as demonstrated by immunocytochemical studies following weight-drop brain injury in rats (Holmin et al., 1995). Early accumulation of neutrophils or polymorphonuclear leukocytes following TBI in rats has been directly linked to the development of cerebral edema (Schoettle et al., 1990; Biagas et al., 1992). Accumulation of leukocytes after TBI contributes to the secondary injury including reduced cerebral blood flow, increased edema and elevated ICP (Zhuang et al., 1993). Activated macrophages that cross BBB and activated microglia within the brain have been indicted as the main culprits in causing progressive neurodegeneration after brain trauma, because they release cytotoxic agents including oxygen free radicals and inflammatory cytokines (Thomas, 1992; Kreutzberg, 1996; Popovich et al., 1997).

Platelet activating factor produced by various cells such as platelets, neutrophils, monocytes, macrophages, neurons, and endothelial cells may enhance BBB permeability and constriction of cerebrovascular system (Armstead et al., 1988; Kochanek et al., 1988). The toxic role of platelet activating factor has been described in neuropathological processes (Kornecki and Ehrlich, 1988; Frerichs and Feuerstein, 1990). Because the platelet activating factor increases intracellular free Ca^{2+} levels in cultured neurons, it may contribute to Ca^{2+} mediated neuronal death following TBI.

Tissue damage in TBI may cause migration and adhesion of leukocytes to the endothelium with upregulation of intracellular adhesion molecule-1 (ICAM-1), which is a member of the immunoglobulin supergene family. Following TBI in rats, upregulation of ICAM-1 has been reported in cerebral microvessels (Isaksson et al., 1997) and suggested to cause sensorimotor deficit (Rancan et al., 2001). Expression of ICAM-1 in astroglia following brain injury in rats required participation of IL-1ß (Shibayama et al., 1996). However, a recent study found that treatment of TBI rats with the monoclonal antibodies to ICAM-1 did not significantly change the functional or histopathological outcome (Isaksson et al., 2001). Complement system has also been suggested to play a prominent role in causing neurodegeneration after TBI (Mollnes and Fosse, 1994). An increase in complement mRNA has been reported following experimental brain lesioning (Johnson et al., 1992).

Anti-inflammatory strategies have recently been initiated in the treatment of TBI. The accumulation of neutrophils has been successfully inhibited by treatment with soluble complement receptor-1 after TBI in rats (Kaczorowski et al., 1995). Administration of the IL-1 receptor antagonist provided neuroprotection reducing the extent of neuronal damage after fluid-percussion brain injury in rats (Toulmond and Rothwell, 1995). The inhibition of biosynthesis or activity of TNF-α in the brain could significantly reduce cerebral edema and improve motor function in rats with weight-drop brain injury (Shohami et al., 1996). Although monoclonal antibodies against ICAM-1 have been shown to be effective in reducing edema and neurodysfunctions in ischemia (Clark et al., 1991; Zhang et al., 1994) and spinal cord injury (Hamada et al., 1996), their efficacy in TBI has not yet been tested. Treatment with IL-10 has been reported to enhance neurological recovery following TBI (Knoblach and Faden, 1998). Blockade of P-selectin has been reported to reduce probe trial

performance on a Morris water maze task following TBI (Grady et al., 1999). Systemic administration of a high, but not a low dose of IL-1 receptor antagonist has been reported to attenuate neurological recovery after TBI (Sanderson et al., 1999). The same study observed that motor function was impaired by the high dose of IL-1 receptor antagonist (Sanderson et al., 1999). Administration of anti-CD11b, a monoclonal antibody directed against the leukocyte adhesion molecule CD11b, reduced neurophil influx after TBI, but did not improve function (Weaver et al., 2000). Nitric oxide (NO) biosynthesized by the inducible NO synthase (iNOS) is an inflammatory product implicated both in secondary damage and in recovery from brain injury. Rats treated with iNOS inhibitors aminoguanidine and L-N-iminoethyl-lysine exacerbated functional outcome and histological damage (Sinz, et al., 1999), thereby suggesting a beneficial role for iNOS in TBI.

It should be noted that most of the TBI studies suggested involvement of cytokines in mediation of neurotoxic effects, while some CNS injury studies reported the participation of cytokines in neuronal survival (Brenneman et al., 1992) and neuroprotection (Hori et al., 1996). Administration of recombinant IL-6 has been claimed to be neuroprotective after permanent focal cerebral ischemia in rats (Loddick et al., 1998). Mice lacking TNF receptors were highly susceptible to cerebrotoxic and ischemic brain injury, suggesting a neuroprotective role for TNF (Bruce et al., 1996). Pre-treatmet with TNF-α has subsequently been shown to be neuroprotective in focal cerebral ischemia in mice (Nawashiro et al., 1997). Other neuroprotective effects of cytokines may be observed in stimulation of astrocyte proliferation, inhibition of Ca^{2+} influx, induction of macrophage host-defense mechanism, and stimulation of neurotrophic factor biosynthesis (Rothwell and Relton, 1993).

Repair-regeneration

In response to traumatic CNS injury, probably an attempt is made to activate repair mechanisms and stimulate neuroregeneration (Varon et al., 1991). The recovery from traumatic injury may be facilitated with the presence of peptide neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT-3). Readily available neurotrophic factors following TBI may support neuronal survival, stimulate neurite sprouting (neuronal plasticity), induce neuronal repair, and re-establish functional connections in the brain. Acute increase in NGF concentrations has been reported after penetrating brain injury (Nieto-Sampedro et al., 1982), cortical ablation (Whittemore et al., 1985), or deafferentation (Needels et al., 1986). Induction in mRNA expression of NGF receptor (NGFR) has been associated with neuronal survival and plasticity following fimbria-formix and angular bundle transections (Gibbs et al., 1991). A recent study has suggested that astrocytes are the major source of NGF upregulation following TBI in rats (Goss et al., 1998). Although a marked increase in expression of NGF at the mRNA and protein levels occurred in acute traumatic phase after cortical contusion injury (DeKosky et al., 1994), a significant loss of NGFR immunoreactive neurons occurred in chronic traumatic phase after fluid-percussion brain injury (Leonard et al., 1994). Hippocampal mRNA expression of BDNF was significantly increased in the dentate gyrus at 3 hours, whereas mRNA expression of NT-3 was decreased in the dentate gyrus at 6 and 24 hours after experimental brain injury in rats (Hicks et al., 1997). Following severe controlled cortical impact injury, NGF and BDNF mRNAs were early, transiently and significantly upregulated while ciliary neurotrophic factor (CNTF) was slow and less amplified in both the area of the lesion and a remote region (Oyesiku et al., 1999). Their respective receptors were also analyzed showing that trkA and trkB mRNAs were significantly elevated while CNTF receptor α (CNTFRα) was significantly downregulated. An increase in GDNF has been demonstrated following destruction of dorsal hippocampus (Bakhit et al., 1991).

Therapeutic strategies with the administration of neurotrophic factors following TBI have been found to be neuroprotective. Treatment with NGF prevented

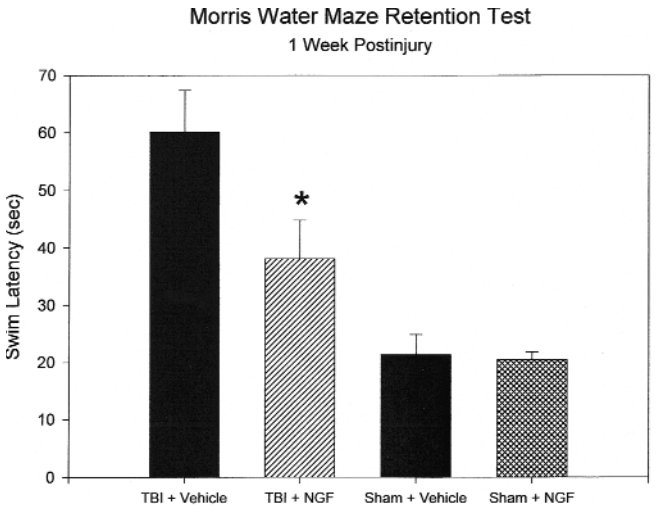

Fig. 2. The effects of intraventricular NGF treatment on spatial memory retention after TB in rats. A bar graph with swim latencies (± SEM) of animals is shown to find a submerged hidden platform. Animals were trained before TBI and retested one week after injury. A mini-osmotic pump (Alza Scientific products) was used to deliver vehicle (artificial cerebrospinal fluid) or vehicle with NGF (2.5S form, 25 μg/ml) to the animals. Vehicle-treated TBI animals (n=10) had longer swim latencies to find the hidden platform compared to sham groups (n=8) indicating that TBI produced spatial memory retention deficits. NFG-treated TBI animals (n=10) had significantly (*, $p<0.05$) shorter swim latencies than vehicle-treaded TBI animals (n=10) indicating an attenuation of spatial memory retention deficits by NGF treatment. (Modified from Dixon et al., 1997).

cholinergic neuronal death in hippocampus (Kromer, 1987). Central infusion of NGF could reduce the extent of apoptotic cell death in septal cholinergic neurons and improve cognitive function (Sinson et al., 1997) and to restore cholinergic neurotransmission deficits (Dixon et al., 1997) following experimental brain injury. Spatial memory deficit following TBI may be related to decreased capacity of cholinergic neurons to produce acetylcholine. Indeed, our experimental study suggests that spatial memory deficit following TBI in rats is mediated by chronic deficits in cholinergic systems that can be improved by neurotrophic factors such as NGF (Fig. 2). Central infusion of BDNF attenuated neuronal cell death following selective brain injury in rodent (Hofer and Barde, 1988). Thus studies in animal models suggest that therapeutic potential of neurotrophic factors should be evaluated in clinical trials for neuroprotection in TBI.

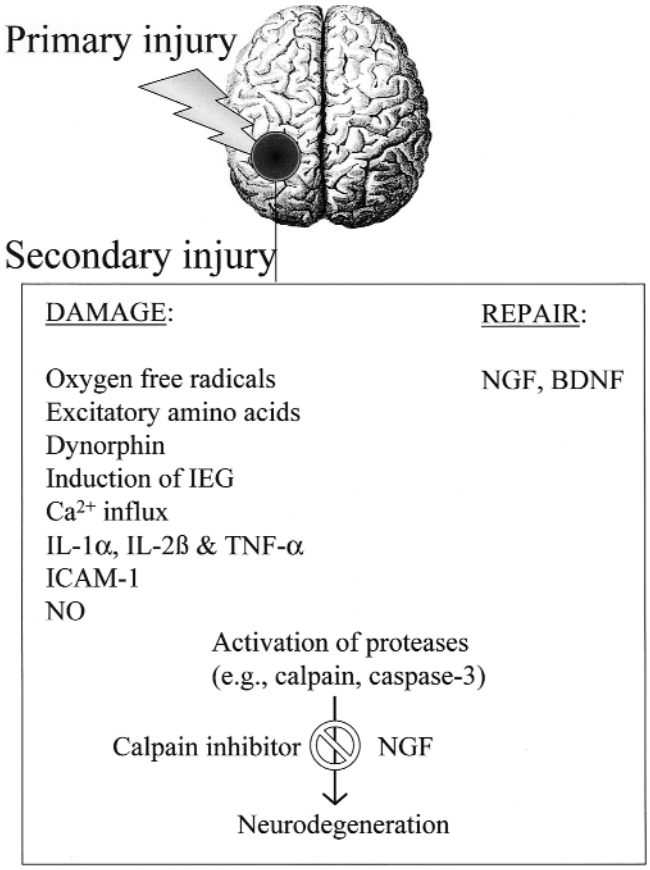

Fig. 3. A simplified schematic presentation of molecular mechanisms in the pathogenesis of TBI and strategy for inhibition of neurodegeneration. The primary injury to the brain initiates the secondary injury process for the pathogenesis in TBI. Various damage inflicting secondary mediators are inter-related promoting the pathogenesis of TBI. Limited number of neurotrophic factors (e.g., NGF, BDNF) may contribute to the repair process but fail to prevent progression of pathogenesis. Thus, the secondary injury process continues ultimately to activate cysteine proteases such as calpain and caspase-3 leading to neuronal death and dysfunction, which are significantly prevented by therapeutic interventions with calpain inhibitor (AK295 or SJA6017) and NGF in animal models.

Conclusion

Investigations in animal models revealed distinct cellular and molecular events, which contribute to the pathogenesis, neurodegeneration, motor dysfunction, and cognitive deficits following TBI. It appears that precautions and preventive measures may dramatically reduce the risk of mechanical primary injury to the brain. A simplified schematic presentation shows several known molecular mechanisms in the pathogenesis of TBI (Fig. 3). Secondary injury cascades are certainly responsible for progression of pathophysiological processes after TBI. The inter-relationships among diverse destructive mediators of the secondary injury make it easy to enhance the neuronal damage and death, while a limited number of repair and regeneration activities ultimately cannot win to prevent the pathophysiological processes (Fig. 3). Thus, therapeutic intervention is a necessity to avoid neurodysfunction and ameliorate pain following TBI. It is important to make appropriate therapeutic interventions as early as possible. A therapeutic agent targeted to a single factor or pathway of secondary injury may not provide enough neuroprotection, especially if the therapy begins in the chronic phase of brain trauma. More than one therapeutic agent may be considered in order to inhibit progressive neurodegeneration resulting from the actions of various mediators of secondary injury at a later stage. Experimental studies suggest that cysteine protease inhibitors in combination with neurotrophic factors may inhibit neurodegeneration and reduce cognitive deficits in TBI. However, the search continues to find out the appropriate therapeutic agents for providing neuroprotection in TBI (Faden, 2001). Time is an important issue in the treatment of TBI. Therefore, future investigations should be focused on identification of timing of specific cellular and molecular events that lead to delayed neuronal death and dysfunction. It is anticipated that further development of novel therapeutic agents based on the latest research knowledge should help the treatment of TBI in the 21st century.

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