Neuroprotection in traumatic brain injury

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The management of traumatic brain injury (TBI) is challenging and there is a need for neuroprotective therapies. A better understanding of the pathomechanism of TBI, particularly of the evolution of secondary damage, is providing targets for new approaches and selected ones in clinical development are described. Clinical trials that have been discontinued in the past for lack of efficacy or other reasons are also listed. One of the problems has been the translation of promising animal experimental results into clinically successful therapies. The complexity of sequelae of TBI requires a multifaceted approach. In addition to the investigation of drugs for neuroprotective effect in TBI, new technologies based on cell/gene therapies, biomarkers and nanobiotechnology are being employed for the integration of neuroprotection with neuroregeneration and are promising.

Introduction

Traumatic brain injury (TBI) is the term applied to brain injury caused by external physical trauma. Brain damage as a cause of death or persistent disability is a major health problem worldwide. The Iraq War since 2002 has produced many cases of TBIs in US soldiers. With protective gear and improved emergency care, the survival rate is higher than in previous wars although there is significant neurological disability among the survivors [1]. The mechanism of TBI has changed from penetrating missile injuries to blast injuries owing to roadside bomb explosions. The mechanism of TBI has an important bearing on neuroprotective measures.

Pathophysiology of TBI

At the cellular level, TBI causes disruption of the neuronal cytoskeleton. This leads ultimately to irreversible division of the axon over a 12-hour period. Microdialysis studies in humans have detected very high concentrations of extracellular glutamate after brain injury. This injures neighboring cells and leads to a cascade of cell death and progressive release of excitotoxic molecules. The sequence of events following TBI is shown in Fig. 1. Multiple mechanisms/pathways involved in damage to the brain indicate that strategies combining more than one agent may be required for neuroprotection in TBI.

Immediate damage and edema following TBI

TBI has long been thought to evoke immediate and irreversible damage to the brain. One major event taking place at the moment of TBI is the massive ionic influx referred to as traumatic depolarization. Excitatory amino acids may play a vital role in this depolarization. This represents one of the most important mechanisms of diffuse neuronal cell dysfunction and damage associated with TBI.

Cerebral edema and associated increased intracranial pressure are the major immediate consequences of TBI that contribute to most early deaths. An important component of brain edema after TBI is astrocyte swelling (cytotoxic edema). Experimental studies have demonstrated that direct mechanical injury to cultured astrocytes produces cell swelling within a few hours, which can be ameliorated by blockade of oxidative/nitrosative stress [2].

Delayed damage following TBI

There are at least two kinds of delayed and progressive pathobiological changes induced by TBI. One of these is axonal damage, which is not the direct consequence of traumatic tissue tearing. Rather, it is the results from complex axolemmal or cytoskeletal changes, or both, which lead to cytoskeletal collapse and impairment of axoplasmic transport. The other change is traumatized brains

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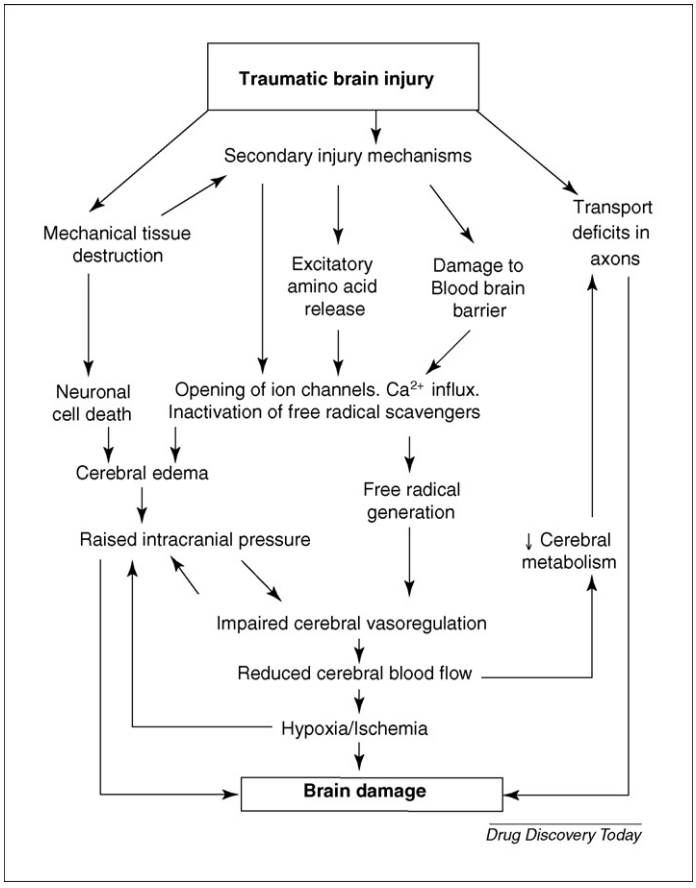

FIGURE 1

Sequence of events following traumatic brain injury.

increased sensitivity to secondary ischemic insult, which is triggered by neurotransmitter storm evoked by TBI. This relatively prolonged (>24 hours) brain hypersensitivity offers a potential window for therapeutic intervention.

Mechanical tearing of axons is unlikely to be the sole cause of axonal damage and structural damage (proteolysis of the cytoskeleton leading to loss of microtubules) appears not to be the cause of disruption of axonal transport [3]. While a subpopulation of injured axons do demonstrate loss of microtubules arising from mechanoporation, the majority of large caliber myelinated axons only demonstrate transport deficits as indicated by focal accumulations of amyloid precursor protein, which is retrogradely transported. Experimental animals that sustain a single episode of a mild, concussive brain injury demonstrate anatomic evidence of axonal injury. Repeated exposures to this mild injury result in increased density of injured axons and an increased extent of axonal damage [4].

Secondary damage in TBI is influenced by changes in cerebral blood flow (CBF), cerebral metabolic dysfunction and inadequate cerebral oxygenation. Excitotoxic cell damage and inflammation may lead to apoptosis. An understanding of the multidimensional cascade of secondary brain injury enables the development of therapeutic options [5].

Blood–brain barrier damage after TBI

The blood–brain barrier (BBB) is disrupted in acute severe TBI. Glut1 density observed in capillaries from acutely injured brain occurs concomitantly with compromised BBB function. Vascular endothelial growth factor appears to be increased in brain tissue during cerebral trauma, and it also increases the permeability of the BBB via the synthesis and release of nitric oxide. After the initial trauma, secondary neuronal injury is associated with a neuroinflammatory response, characterized by microglial and astrocytic activation, resulting in the release of reactive oxygen species and inflammatory cytokines. These events result in BBB breakdown and the development of cerebral edema [6].

Because many signaling cascades are initiated immediately after the traumatic event, differential protein expression may be involved in BBB function. At acute time points postinjury, altered protein expression may result from altered translation efficiency or turnover rate, rather than from a genomic response. The application of tandem 2D gel electrophoresis and mass spectrometry analysis is a powerful approach for directly screening differential protein expression following TBI. The use of this technique revealed significantly increased cyclophilin A levels in isolated brain microvessels 30 min after injury [7]. Postinjury administration of cyclosporine A significantly attenuated BBB permeability measured 24-hour postinjury, suggesting cyclophilin activity after TBI may be detrimental. Direct injection of purified recombinant cyclophilin A, however, attenuated both BBB permeability and tissue damage in a stab wound model of injury. These findings suggest that increased expression of cyclophilin A may play a protective role after TBI, whereas other cyclophilin isoforms may be detrimental.

Biomarkers of TBI relevant to neuroprotection

Although there are clinical biomarkers of TBI, such as seizures and impairment of consciousness as well as brain imaging, no definitive diagnostic test for TBI is available to physicians to determine the seriousness of injury or the extent of cellular pathology. There is a need for discovery and validation of better biomarkers for TBI. For a comprehensive evaluation of TBI, multiple biomarkers need to be correlated. These include biomarkers in cerebrospinal fluid (CSF) and blood, in addition to brain imaging, neurophysiological studies (electroencephalography and evoked potentials) and tests of cognitive function. Neuroproteomic technologies are uniquely suited for the discovery of otherwise unnoticed TBI biomarkers and are being used for mapping changes in proteins after injury. This will be very useful for developing diagnostic predictors after TBI and for identifying new therapeutic targets.

CSF C-tau levels are elevated in patients with TBI. C-tau is a reliable, quantitative biomarker for evaluating TBI-induced neuronal injury and a potential biomarker of efficacy of neuroprotective drug in the rat TBI model [8]. Serum biomarkers of TBI may have clinical utility in stratifying injury severity level, predicting adverse secondary events or outcomes, and monitoring the effectiveness of therapeutic interventions. Serum biomarkers may be useful for predicting secondary pathologies such as elevated intracranial pressure associated with TBI [9].

Genetic influences on outcome following TBI

Several genes have been implicated by influencing the outcome following TBI. Currently, the most extensively studied gene has been *apoe*. It can influence overall and rehabilitation outcome, coma recovery, risk of post-traumatic seizures, as well as cognitive

BOX 1

Current conventional management of traumatic brain injury

General supportive care of various body functions
Reduction of increased intracranial pressure and cerebral edema
Surgical decompression
Removal of hematomas and foreign bodies
Osmotic agents
Neuroprotection
Pharmacological approaches
Nonpharmacological approaches
Management of late sequelae: for example seizures and behavioral disorders
Rehabilitation for neurological disability
Physical therapy
Mental training

and behavioral functions following TBI. Pathologically, *apoe* is associated with increased amyloid deposition, amyloid angiopathy, larger intracranial hematomas and more severe contusional injury. The proposed mechanism by which *apoe* affects the clinicopathological consequences of TBI is multifactorial and includes amyloid deposition, disruption of cytoskeletal stability, cholinergic dysfunction, oxidative stress, neuroprotection and central nervous system plasticity in response to injury. Other putative genes have been less extensively studied and require replication of the clinical findings. The *comt* and *drd2* genes may influence dopamine-dependent cognitive processes such as executive/frontal lobe functions. Inflammation, which is a prominent component in the pathophysiological cascade initiated by TBI, is in part is mediated by the interleukin genes, while apoptosis that occurs as a consequence of TBI may be modulated by polymorphisms of the *p53* gene. The *ace* gene may affect TBI outcome via mechanisms of CBF and/or autoregulation and the *cacna1a* gene may exert an influence via the calcium channel and its effect on delayed cerebral edema. Although several potential genes that may influence outcome following TBI have been identified, future investigations are needed to validate these genetic studies and identify new genes that might influence outcome following TBI [10].

Management of TBI

Current management of TBI is multidisciplinary and various measures are shown in Box 1. Control of cerebral edema and raised intracranial pressure are the most crucial problems in the management of acute severe TBI.

Control of intracranial pressure and cerebral edema

The full extent of recovery from injury can be improved by controlling edema, but the outcome is not predictable. Current treatments for cerebral edema are very limited and include osmotherapy by the administration of hypertonic mannitol or hypertonic saline and, in very severe cases, by surgical decompression. The beneficial effects of osmotherapy are limited and are often not successful, because osmotherapy, by withdrawing water, shrinks healthy parts of the brain along with the damaged area. Glucocorticoids have not been very successful in the treatment of most forms of edema. These conventional treatment of cerebral edema needs to be improved. Effective control of cerebral edema is essential for neuroprotection following injury. Hyperbaric oxygen (HBO) therapy, an effective treatment for cerebral edema, means the administration of oxygen at pressures is greater than the atmospheric pressure at sea level. The use of HBO and its rationale for the management of TBI has been reviewed elsewhere [11]. Oxygen at 1.5–2 ATA (atmospheric pressure absolute) reduces cerebral edema by vasoconstriction but prevents cerebral ischemia by high oxygen tension. HBO modifies several pathophysiological changes in TBI and reduces intracranial pressure. The practicality of this method is limited by the availability of hyperbaric chambers.

Neuroprotection in TBI

Various investigational neuroprotective strategies for TBI are listed in Table 1. These strategies have been described in detail in a special report on this topic [12]. A few will be discussed briefly in the following text.

Citicoline for neuroprotection in TBI

Citicoline (cytidine-5′-diphosphocholine) is a form of an endogenous compound, which is used for getting more choline into the brain when the requirements are high. Upon oral or parenteral administration, citicoline releases its two principle components, cytidine and choline. Once absorbed, the cytidine and choline disperse widely throughout the organism, cross the BBB and reach the CNS, where they are incorporated into the phospholipid fraction of the membrane and microsomes. Phase III clinical trials failed to demonstrate efficacy as a neuroprotective agent in stroke. In a rat model of TBI, citicoline was shown to prevent neuronal loss in the hippocampus, decreased cortical contusion volume and improved neurological recovery [13]. It is in phase III clinical trials for TBI.

Cyclosporin for neuroprotection in TBI

Neuroprotection by cyclosporin A, an immunosuppressant, results from the inhibition of calcineurin and protection from mitochondrial damage caused by the formation of a mitochondrial permeability transition pore induced by cyclophilin D, one of the prolyl *cis*/*trans* isomerase family members [14].

NeuroSTAT® NeuroVive Pharmaceutical AB/Maas Biolab), a patented cyclosporin neuroprotection formula, has been tested in models of military TBI and nerve gas poisoning in collaboration with the Walter Reed Army Institute of Research (WRAIR), USA. The unique WRAIR double-insult model of brain trauma and simultaneous hypoxia closely simulates the real life battlefield-injured soldier who may have a delay before reaching advanced medical care. Initial work by WRAIR indicates that NeuroSTAT® is highly neuroprotectant. Brain function, memory, learning and retention rates are dramatically improved by cyclosporin treatment in animal models of TBI. Cyclosporin is a mitochondrial neuroprotectant that can cross the BBB of the cerebrum injured by projectile, blast and blunt force. NeuroSTAT® could be administered by intravenous injection to brain injured soldiers by first-responder battlefield paramedics to stop further brain damage.

Erythropoietin for neuroprotection in TBI

Erythropoietin (EPO) and its receptor function because primary mediators of the normal physiological response to hypoxia and

TABLE 1

Neuroprotective strategies for traumatic brain injury.

Pharmacological targeting of various mechanisms for neuroprotection
AMPA-receptor antagonists: for example zonampanel
Antiepileptic drugs: for example topiramate, levetiracetam
Anti-inflammatory agents: COX-2 inhibitors, minocycline
Antioxidants: for example ebselen, allopurinol (xanthin oxidase inhibitor)
Apoptosis inhibitors: for example erythropoietin
Bradykinin antagonists: for example Anatibant
Immunophilin ligands: for example cyclosporine A, FK506
Ion channel blockers: for example calcium channel antagonists
Necrosis inhibitors: for example calpain inhibitors
Neurotrophic factors: for example nerve growth factor, brain-derived neurotrophic factor
Neurotrophic factor analogs: for example NNZ-2566 (Neuren Pharmaceuticals)
Neurosteroids: for example estrogen, progesterone
NMDA receptor antagonists: for example traxoprodil, aptiganel, eliprodil, memantine and arcain
Non-NMDA antagonists: NBQX (Novo Nordisk)
Nitric oxide modulators
Oxygen carriers: for example Oxycyte™ (Synthetic Blood International Inc.)
Polyethylene glycol (PEG)
Thyrotropin-releasing hormone analogs
Pharmacological neuroprotective agents: multiple or undefined mechanisms
Barbiturates
Cannabinoids: for example dexanabinol (Pharmos), KN38-7271 (KeyNeurotek)
Citicoline (CerAxon)
Glutathione
Corticosteroids
Creatine
Magnesium sulfate
Omega-3 fatty acids
Nanobiotechnology-based neuroprotection in TBI
Innovative biological approaches for neuroprotection
Antisense approaches
Cell/gene therapy
Vaccines
Nonpharmacological approaches
Deep brain stimulation
Hyperbaric oxygen therapy
Hypothermia

the transcription factor hypoxia-inducible factor-1 (HIF-1) upregulates EPO following hypoxic stimuli. Recombinant human EPO (rhEPO) is approved by the FDA for the treatment of anemia that may result from a variety of conditions, including the anemia associated with chronic renal failure. EPO is expressed in the human CNS and has demonstrated remarkable neuroprotective potential in cell culture and animal models of disease. The exact mechanism of neuroprotective effect of EPO is not clear but multiple actions may contribute to neuroprotection, for example as a neurotrophic, antioxidant or anti-inflammatory agent. In a rat model of TBI, the administration of rhEPO protects neurons by enhancing *Bcl-2* expression, thereby inhibiting TBI-induced neuronal apoptosis [15]. rhEPO also reduces cerebral edema and promotes the recovery of motor deficits [16]. Currently, rhEPO is in phase II/III clinical trials for TBI. The hypothesis for another phase II clinical trial on severe TBI patients with another EPO preparation, darbepoetin alfa (Aranesp®), is based on the reduction of CSF levels of glutamate within a 96-hour period after TBI. This effect is potentially mediated through the activation of EPO receptors, which prevents the exocytosis of glutamate, a known neurocytotoxin, into CSF.

KN 38-7271

KN 38-7271 (formerly BAY 38-7271) is a structurally novel, selective and highly potent cannabinoid CB1/CB2 receptor agonist *in vitro* and *in vivo* with pronounced neuroprotective efficacy in a rat TBI model, showing a therapeutic window of at least five hours [17]. Furthermore, neuroprotective efficacy was also found in models of transient and permanent occlusion of the middle cerebral artery and brain edema models as well. The neuroprotective efficacy of BAY 38-7271 is mediated by multiple mechanisms triggered by cannabinoid receptors. In phase I studies, KN 38-7271 was safe and well tolerated when administered by intravenous infusion for either 1 or 24 hours. Because the doses of KN 38-7271 in animals needed for maximal neuroprotective efficacy were significantly lower than those inducing typical cannabinoid-like side effects, it is to be expected that the compound will offer a novel therapeutic approach with a favorable therapeutic window for the treatment of TBI or cerebral ischemia. It is scheduled for phase II clinical trials by KeyNeurotek AG.

Neurosteroids as neuroprotective agents for TBI

Naturally occurring neurosteroids are potent allosteric modulators of the $GABA_A$ receptor and through augmentation of this receptor function can protect neuronal cells against NMDA over-activation, ischemia and TBI [18]. Neurosteroids, such as progesterone and its metabolite, allopregnanolone (5α-pregnan-3β-ol-20-one), have been shown to reduce the expression of inflammatory cytokines in the acute stages of TBI, although how they do this is not completely understood. In one study both progesterone and allopregnanolone treatments were shown to enhance the production of CD55 following contusion injuries of the cerebral cortex in rats [19]. CD55, a single-chain type 1 cell surface protein, is a potent inhibitor of the complement convertases which are activators of the inflammatory cascade. The increased expression of CD55 could be an important mechanism by which steroids help to reduce the cerebral damage caused by inflammation.

Progesterone appears to exert its neuroprotective effects in TBI by protecting or rebuilding the BBB, decreasing the development of cerebral edema, down-regulating the inflammatory cascade, and limiting cellular necrosis and apoptosis [20]. The study of enantiomer of progesterone (ent-PROG) at the PROG receptor (PR) shows that the treatment *in vivo* decreases cerebral edema, cell death mediators, inflammatory cytokines and reactive gliosis, and increased antioxidant activity [21]. These findings suggest that the progestin-mediated prosurvival response seen with TBI is

regulated either independently of the classical PR or via nongenomic PR-regulated actions. In aged male rats, the treatment with progesterone improves short-term motor recovery and attenuates edema, secondary inflammation, and cell death after TBI [22]. A phase II, randomized, double-blind, placebo-controlled clinical trial was conducted to assess the safety and potential benefit of administering progesterone to patients with acute TBI [23]. No serious adverse events were attributed to progesterone. Thirty days postinjury, the majority of severe TBI survivors in both groups had relatively poor Glasgow Outcome Scale-Extended and Disability Rating Scale scores. However, moderate TBI survivors who received progesterone were more likely to have a moderate to good outcome than those randomized to placebo.

In addition to intracellular PRs, the membrane-binding sites of progesterone may be involved in neuroprotection. The first putative membrane receptor of progesterone (distinct from the classical intracellular PR isoforms) with a single membrane-spanning domain, has been cloned from porcine liver. It is referred to as progesterone-binding protein, 25-Dx [24]. The distribution and regulation of 25-Dx in the nervous system may provide some clues to its functions. In the brain, 25-Dx is particularly abundant in the hypothalamic area, circumventricular organs, ependymal cells of the ventricular walls, and the meninges. It is coexpressed with vasopressin in neurons of the paraventricular, supraoptic and retrochiasmatic nuclei. In response to TBI, 25-Dx expression is up-regulated in neurons and induced in astrocytes. The expression of 25-Dx in structures involved in CSF production and osmoregulation, and its up-regulation after brain damage, point to a potentially important role of this progesterone-binding protein in the maintenance of water homeostasis after TBI. These observations suggest that progesterone's actions may involve different signaling mechanisms depending on the pathophysiological context, and that 25-Dx may be involved in the neuroprotective effect of progesterone in TBI.

Neurotrophic factors for TBI

Injured neurons may suffer three potential fates: death, persistent atrophy or recovery. The ability of injured neurons in the adult brain to recover from injury depends on the expression of growth-related genes and the responsiveness to survival and growth signals in the environment. These signals include neurotrophic factors (NTFs) and substrate molecules that promote neurite growth. The specificity of neurotrophic responsiveness parallels patterns observed during the development of the nervous system.

The poor ability of the CNS to cause axons to regenerate after injury has been partly attributed to astrocyte behavior after injury, which is manifested by their limited ability to migrate and repopulate the injury site. Astrocyte migration is promoted by various NTFs suggesting that the appropriate choice of growth factors at the appropriate postinjury period may compensate for the glial support deficiency or the presence of glial inhibitory factors in CNS.

One of the problems with neurotrophic factor administration is the penetration of the BBB. Breach of BBB has been demonstrated in rats following cortical contusions. This indicates that regions that are not initially destroyed by cortical impact but have breached BBB may be accessible to NTFs administered intravenously both immediately and days after brain trauma. Cell and gene therapies provide more effective methods of administration of NTFs.

Various studies of NTFs in TBI have shown that they have a neuroprotective action and enhance cellular systems involved in the maintenance of Ca^{2+} homeostasis and free radical metabolism. The neuroprotective efficacy of intracerebral nerve growth factor (NGF) infusion has been demonstrated during the acute phase of experimental head injury. This beneficial effect of NGF may be related to its ability to attenuate traumatically induced apoptotic cell death.

NNZ-2566 (Neuren Pharmaceuticals). This is a small molecule analog of Glypromate® (Glycine-Proline-Glutamate, derived from IGF-1) with good oral bioavailability and up to ten times the potency of Glypromate®. It is being developed for several acute, subacute and chronic conditions including acute and recovery phase treatment of TBI. Animal experimental studies have shown that it can reduce nonconvulsive seizures, which are a significant predictor of outcome of TBI. They are easily detected and provide an additional endpoint in clinical trials. NNZ-2566 is in phase II clinical trials.

Nanobiotechnology-based neuroprotection in TBI

One of the important applications of nanobiotechnology is the regeneration and neuroprotection of the CNS that will have a significant impact on TBI [25]. It will also improve study of pathomechanisms of TBI. Quantum dot (QD) technology can be used to gather information about how the CNS environment becomes inhospitable to neuronal regeneration following injury by studying the process of reactive gliosis. Glial cells, housekeeping cells for neurons, have their own communication mechanisms that can be triggered to become reactive following injury. Nanoparticles composed of cerium oxide or yttrium oxide protect nerve cells from oxidative stress and the neuroprotection is independent of particle size [26]. Poly(D,L-lactide co-glycolide; PLGA) nanoparticles loaded with superoxide dismutase have neuroprotective seen up to six hours after H_2O_2-induced oxidative stress, which appears owing to the stability of the encapsulated enzyme and its better neuronal uptake after encapsulation [27].

The peptide nanofiber scaffold is an effective technology for tissue repair and restoration and is a promising treatment of TBI. This peptide nanofiber scaffold has several advantages over currently available polymer biomaterials. The network of nanofibers is similar in scale to the native extracellular matrix and thus provides an environment for cell growth, migration and differentiation. This peptide disintegrates and is immunologically inert. Nanoparticles can improve drug delivery to the CNS and facilitate crossing of the BBB and more precisely target a CNS injury site. A combination of nanofibers to repair the damage and neuroprotective drugs to prevent further damage may provide an effective combined approach for managing TBI [28].

Cell therapy for TBI

Stem cell based cellular replacement strategies have potential therapeutic role following TBI but the mechanism by which stem cells produce their effect, for example via integration into surviving neuronal circuits, local neurotrophic support or modification of the local microenvironment to enhance endogenous regeneration and neuroprotection remains to be assessed further [29]. Transplantation of bone marrow derived mesenchymal stromal cells (MSCs) into the injured brain has potential therapeutic benefit. Although it has been suggested that differentiation of

MSCs into cells of neural lineage may occur, this is unlikely to be a major factor in functional recovery after TBI, but other mechanisms that may play a role include neuroprotection, creation of a favorable environment for regeneration, expression of growth factors or cytokines [30].

Preliminary discoveries of the efficacy of cell therapy are currently being translated into clinical trials. A phase I clinical trial for evaluating the use of autologous bone marrow derived mononuclear cells to treat children with isolated severe TBI is ongoing at the Memorial Hermann Children's Hospital of University of Texas Medical School and results are expected to be available by the end of 2008 or early 2009 [31]. The clinical trial is based on laboratory and animal research indicating that stem cells can migrate to an injured area of the brain, differentiate into new neurons and support cells and induce brain repair.

Gene therapy for TBI

Neural stem cells have been retrovirally transduced to produce NGF and transplanted into the injured brain with marked improvement of cognitive and neuromotor function and rescue of hippocampal CA3 neurons during the acute post-traumatic period. The clinical implication of this is that NGF gene transfer can provide neuroprotection. Human Ntera-2 neurons genetically modified to express NGF significantly attenuate cognitive dysfunction following TBI in mice indicating that this is a practical and effective method for stable *ex vivo* gene delivery into the CNS [32].

A review of the currently available information on preclinical studies reveals that there are several gene targets with therapeutic potentials and vectors that can be used to deliver the candidate genes [33]. In spite of obstacles in translating these techniques into effective gene therapy in humans, they provide new strategies for neuroprotection in TBI.

Vaccine for TBI

Current evidence suggests that the injured CNS can benefit from autoimmune manipulations. Active or passive immunization with CNS-associated selfantigens has been shown to promote recovery from a CNS insult. This beneficial 'autoimmunity' is not solely an outcome of immune manipulation, but is also a physiological response, evoked by a nonpathogenic insult and apparently designed to counteract the insult-related toxicity which is induced in part by essential physiological compounds present in excess of their normal levels. It appears that when the buffering capacity of constitutive local mechanisms (transporters, enzymes and so on) that normally regulate these compounds is exceeded, assistance is recruited from the immune system. Like the overactive physiological compounds themselves, the immune system needs to be rigorously regulated to produce adequate phagocytic activity and the required quantity of cytokines and growth factors at the right time and place. Boosting of this autoimmune response by vaccination is potentially a powerful strategy for neuroprotection in TBI. A vaccine-based approach can be used to promote axonal regeneration and repair following TBI [34].

Clinical trials of neuroprotective agents in TBI

As of June 2008, approximately 150 clinical trials are listed that are relevant to TBI (http://clinicaltrials.gov/search/term=Traumatic%20Brain%20Injury). They cover a wide range of problems associated

TABLE 2

Ongoing or recently completed clinical trials for neuroprotection in TBI.

Agent/sponsor	Mechanism of action	Current status/comments
AL-208/Allon therapeutics	Activity-dependent neurotrophic factor	Phase I
Anatibant (XY2405)/Xytis	Bradykinin B_2 receptor antagonist	Phase II
Citicoline/National Institute of Child Health and Human Development, USA	An endogenous compound, which is used orally or parenterally for getting more choline into the brain when the requirements are high	Phase III
Darbepoetin alfa (Amgen's Aranesp®)/ University of Alberta	Effect is mediated through the activation of epoetin receptors whose activation prevents the exocytosis of glutamate, a known neurocytotoxin, into CSF	Phase II
Recombinant human erythropoietin/ Medical College of Wisconsin	Multiple mechanisms of neuroprotective effect: antiapoptotic and reduction of cerebral edema	Phase II/III
Ketamine/University of Arkansas	Ketamine, an intravenous anesthetic, has possible neuroprotective effect	Phase II in children with TBI scheduled to start in December 2008
KN 38-7271/KeyNeurotek AG	A cannabinoid receptor agonist	Phase I completed scheduled for phase II
NNZ-2566/ Neuren Pharmaceuticals	This is a small molecule analog of Glypromate® (Glycine-Proline-Glutamate, derived from IGF-1)	Completed phase I and phase II scheduled to start in 2008
Oxycyte/Synthetic Blood International Inc.	A second-generation PFC that increases oxygen levels in damaged brain	Phase IIb
Progesterone intravenous/NIND	Reduction of brain swelling and damage after blunt TBI	Phase II ongoing
Rivastimine/Novartis	ChE inhibitor approved for the treatment of Alzheimer's disease, now tested for the treatment of cognitive deficits resulting from TBI	Phase IV, open label extension completed
Stem cell transplantation/University of Texas	Autologous bone marrow derived stem cells for TBI in children	Phase I ongoing

NIND, National Institute of Neurological Disorders and Stroke, USA.

TABLE 3

Discontinued or failed clinical trials for neuroprotection in traumatic brain injury.

Agent/Sponsor	Mechanism of action	Comments
ACEA 1021(licostinel)/NIH	Glycine site glutamate antagonist	Discontinued in phase I
BAY X 3702/Bayer	$5HT_{1A}$ agonist, ion channel blocker	Terminated after phase II
Bradycor/Cortech	Bradykinin receptor antagonist	No benefit shown in phase II controlled trials
Cerestat/Cambridge Neuroscience	Noncompetitive NMDA site glutamate antagonist	Terminated in phase III
Corticosteroids	Neuroprotective and antioxidant	Phase III CRASH (corticosteroids randomization after significant head injury) trial failed to show efficacy
D-CCPene/Novartis	Competitive NMDA antagonist	Completed phase III but discontinued
Dexanabinol/Pharmos	A cannabinoid that inhibits inflammatory reaction in the CNS	Phase III completed but failed to show efficacy
Eliprodil/Synthelabo	Polyamine site glutamate antagonist	Phase II showed no significant effect
Magnesium sulfate/NINDS	Noncompetitive NMDA antagonist	Phase III failed to show efficacy
Nimodipine/Bayer	Calcium channel entry blocker	No significant benefit in TBI
PEG-SOD	Antioxidant	Phase III failed to show benefit
Prednisone/generic	Neurosteroid/neuroprotective	Phase II showed some benefit
Selfotel/Novartis	Competitive NMDA site glutamate antagonist	Terminated in phase III
Tirilazad mesylate/Pfizer	Lipid peroxidation inhibitor	No significant benefit in phase III. Development discontinued for TBI
Traxoprodil (CP101,606)/Pfizer	NMDA N2B subunit receptor antagonist	Phase I in severe TBI did not show definitive evidence of efficacy
Ziconotide (SNX-111)	Non-NMDA antagonist	Discontinued after phase II because of higher mortality in the treated group

NMDA = *N*-methyl-D-aspartate.

with TBI and deal with surgical procedures, prevention of seizures diagnosis, rehabilitation and psycho-social sequelae of TBI. The status of 12 ongoing or completed clinical trials relevant to neuroprotection in TBI is shown in Table 2. Clinical trials that were discontinued owing to lack of efficacy or adverse effects are listed in Table 3.

Failed clinical trials in TBI

As shown in Table 3, approximately 14 clinical trials of therapeutic agents for TBI were discontinued owing to various reasons, which were mostly failure to demonstrate efficacy in human patients, although preclinical studies had shown efficacy in animal models. One of the problems in translating laboratory results into the clinic is the lack of suitable animal models of TBI. Some of the clinical trials were in advanced stages and had completed phase III. The reasons for failure and suggestions for improvements have been discussed elsewhere [12].

Conclusions

Developing neuroprotective drugs for TBI has proved challenging but frustrating over the past decade. Secondary brain damage, following severe head injury is considered to be a major cause for poor outcome. Impressive reductions of the extent of brain damage in experimental studies have raised high expectations for cerebral neuroprotective treatment in the clinic. Therefore, multiple compounds were and are being evaluated in trials. However, the failure rate in clinical trials for neuroprotective therapies of TBI has been high. There is also a need for improving animal models of TBI.

Future advances in the pharmacological treatment of TBI are likely to include the evaluation of sequentially timed therapies combining multiple and targeted agents. Finding effective treatments for TBI is not going to be easy and is evidently going to require numerous clinical trials [35]. New technologies for cell and gene therapy as well as nanobiotechnology will contribute to the development of new therapies for neuroprotection combined with neuroregeneration for injuries of the CNS. Continued research efforts are required to identify and test new neuroprotective agents, to develop a better understanding of the pathophysiologic mechanisms, and to improve the design and analysis of clinical trials [36]. Most recently, different experimental approaches and altered clinical trial methods have provided renewed optimism regarding future trials of neuroprotective agents [37].

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