

Emerging Therapies in Traumatic Brain Injury

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Abstract

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Despite decades of basic and clinical research, treatments to improve outcomes after traumatic brain injury (TBI) are limited. However, based on the recent recognition of the prevalence of mild TBI, and its potential link to neurodegenerative disease, many new and exciting secondary injury mechanisms have been identified and several new therapies are being evaluated targeting both classic and novel paradigms. This includes a robust increase in both preclinical and clinical investigations. Using a mechanism-based approach the authors define the targets and emerging therapies for TBI. They address putative new therapies for TBI across both the spectrum of injury severity and the continuum of care, from the field to rehabilitation. They discuss TBI therapy using 11 categories, namely, (1) excitotoxicity and neuronal death, (2) brain edema, (3) mitochondria and oxidative stress, (4) axonal injury, (5) inflammation, (6) ischemia and cerebral blood flow dysregulation, (7) cognitive enhancement, (8) augmentation of endogenous neuroprotection, (9) cellular therapies, (10) combination therapy, and (11) TBI resuscitation. The current golden age of TBI research represents a special opportunity for the development of breakthroughs in the field.

Despite decades of basic and clinical research, treatments to improve outcomes after traumatic brain injury (TBI) are limited. In severe TBI, with few exceptions, supportive measures such as controlling brain swelling using osmolar therapy or barbiturates, and/or surgical interventions remain the standard of care. In severe TBI, development of novel therapies targeting secondary injury has been the topic of consid-

erable research, but failures in translation continue to mount with the recently published clinical trial on erythropoietin¹ and recent halting of the PROTECT trial of progesterone therapy (personal communication, David Wright, MD). Only treatment with amantadine in the subacute phase has translated—from the controlled cortical impact (CCI) model of TBI in rats to moderate–severe TBI in humans.² In mild TBI

(mTBI), empiric therapies targeting sequelae such as cognitive impairment and posttraumatic stress disorder are often used. However, there has been little research on therapies in mTBI, secondary injury pathways, or the link between mTBI and neurodegenerative disease. Given the increased recognition of the scope of the problem, the growth in funding for TBI research, and the expanding discussion of therapies,³ an acceleration of research into the treatment of TBI across the injury spectrum is emerging and there is cause for optimism. Using a mechanism-based approach we will define the targets and emerging therapies for TBI. We will address emerging therapies for TBI across the spectrum of severity and the continuum of care, from the field to rehabilitation. We will discuss TBI therapy using 11 categories, namely, (1) excitotoxicity and neuronal death, (2) brain edema, (3) mitochondria and oxidative stress, (4) axonal injury, (5) inflammation, (6) ischemia and cerebral blood flow (CBF) dysregulation, (7) cognitive enhancement, (8) augmentation of endogenous neuroprotection, (9) cellular therapies, (10) combination therapy, and (11) TBI resuscitation.

Therapies Targeting Excitotoxicity and Neuronal Death

Excitotoxicity and its link to neuronal death pathways in TBI has been richly explored, and yet after 45 years of research most antiexcitotoxic therapies have failed to translate.^{4,5} New understanding of glutamatergic neurotransmission inspires optimism that future drugs will be more effective.

Excitotoxicity after Traumatic Brain Injury

Presynaptic glutamate release depolarizes post-synaptic neurons by opening ion channels such as *N*-methyl-D-aspartate receptors (NMDARs) that permit Na^+ and Ca^{2+} to surge into cells. Excitotoxic events occur early after TBI and trigger apoptosis, necrosis, necroptosis, autophagy, or pyroptosis (none of which are mutually exclusive). Ca^{2+} overload is a key feature that is upstream to cell-death signaling.⁶ Na^+ overload is less significant, but may contribute to neuronal swelling.⁷ After TBI, there is a surge in extracellular glutamate followed by persistent elevations.⁸ Extracellular glutamate increases intracellular Ca^{2+} (iCa^{2+}) by activating neuronal glutamate receptors (GluRs) like NMDARs and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA). Release of Ca^{2+} from intracellular stores in organelles also contributes to excitotoxicity.⁹

Necrosis and Apoptosis

Nuclear Ca^{2+} promotes neuronal survival by inducing protective genes like brain-derived neurotrophic factor (BDNF). In contrast, cytoplasmic Ca^{2+} is toxic. Necrotic death is triggered in part by calpains— Ca^{2+} activated cysteine proteases that destroy survival substrates.¹⁰ Traumatic brain injury induces rapid and sustained iCa^{2+} elevations and calpain activation.^{10,11} Blocking calpain activity in TBI models reduces tissue loss.¹² Increased iCa^{2+} also overwhelms mitochondria resulting in oxidative stress, mitochondrial permeability transition pore (MPTP) opening and cytochrome c release.¹³

This activates the intrinsic pathway (caspase-9 dependent).¹⁴ Apoptotic cell death has been characterized after TBI.^{14,15} Therapies that block executioner caspases reduce tissue loss and improve outcome.^{16,17}

Additional Excitotoxic Death Pathways

Necroptosis (programmed necrosis) is regulated by receptor-interacting serine/threonine-protein kinase (RIPK1/3) and mixed lineage kinase domain-like.¹⁸ Necroptosis is induced by co-incubation of tumor necrosis factor α ($\text{TNF}\alpha$) with Z-VAD-FMK (a pan caspase inhibitor). Alone, $\text{TNF}\alpha$ stimulates the extrinsic cell death receptor-mediated apoptotic pathway (caspase-8 dependent). Caspase-8 cleaves and destroys RIPK preventing its activation.¹⁹ The addition of Z-VAD during $\text{TNF}\alpha$ – induced apoptosis in neurons stabilizes RIPK1/3 activating necroptosis.²⁰ The necroptotic inhibitor necrostatin-1 inhibits neuronal death after TBI.²¹ However, excitotoxicity induces necroptosis in cultured neurons, but accounts for a fraction of the cell death.²² Autophagy is a homeostatic mechanism that regulates catabolism of damaged organelles (macroautophagy). Loss of autophagy may contribute to neurodegenerative disease,²³ whereas overactivation may promote cell death. There is marked upregulation of autophagy after TBI.²⁴ Mice treated with the autophagy inhibitor 3-methyladenine show reduced neuronal death after TBI.²⁵ However, autophagy may also be beneficial late after TBI by helping to “clean-up” injured brain.²⁶ Microglia can also release glutamate, enhance excitotoxicity,²⁷ and promote inflammasome-mediated cell death (pyroptosis) linked to caspase-1. All of these cell death pathways are targets for TBI therapy.

Antiexcitotoxic Therapy

Excitotoxicity plays a key role in tissue damage after TBI. Vespa et al²⁸ reported early posttraumatic subclinical seizure activity and its deleterious effects after severe TBI. Most strategies to reduce excitotoxicity include averting Ca^{2+} accumulation, or inhibiting downstream death signaling (caspases/apoptosis or calpains/necrosis). Historically NMDARs have been key targets. Nonselective NMDAR antagonists like MK801 are neuroprotective in TBI models. However, they failed in clinical TBI due to psychosomatic side effects, inadvertent death of select brain regions (cingulate and retrosplenial cortex), and limited therapeutic window.⁵ Progress in NMDAR-mediated neurotransmission has revealed greater complexity than previously appreciated—and may better inform therapy. *N*-Methyl-D-aspartate receptors consist of heterodimeric glutamate receptor (GluR) subunits including NR1, NR2A, and NR2B. NR2A containing NMDARs are enriched in the synapse (synaptic NMDARs).²⁹ NR2B containing NMDARs are enriched at extrasynaptic sites (extrasynaptic NMDARs).²⁹ Spatial distribution of NMDARs greatly affects excitotoxic signaling. Activation of synaptic NMDARs is neuroprotective. They increase nuclear Ca^{2+} , activate CREB, BDNF, protein kinase B (AKT), phosphorylated-JACOB (pJACOB), and upregulate antioxidants.^{30–33} In contrast, activation of extrasynaptic NMDARs by glutamate spillover after TBI has the opposite effect. Extrasynaptic

NMDARs increase cytoplasmic Ca^{2+} , inhibit CREB, AKT, p-JACOB, BDNF, active calpain, stimulate death-associated protein kinase (DAPK), and activate autophagy.^{30,32–35} Extrasynaptic NMDARs play a role in cell death in TBI. The selective NR2B antagonist Ro 25–6981 inhibits induction of autophagy after TBI.³⁴ Stretch injury increases NR2B/NMDAR currents, which open AMPARs. That deleterious cascade is prevented by the NR2B antagonists Ro 25–6981 and memantine (a lipophilic form of amantadine).³⁶ Memantine (Namenda) was tested in rodent TBI many years ago.³⁷ Recently it was discovered to block extrasynaptic NMDARs while sparing synaptic NMDAR function.³⁸ It is approved by the U.S. Food and Drug Administration (FDA) to treat dementia and has proved a more tolerable NMDAR antagonist than MK801.³⁹ Memantine and other next-generation NR2B-selective antagonists deserve additional study.⁴⁰

Considerations for Chronic Recovery after Traumatic Brain Injury

Long-term blockade of NMDARs may limit recovery of brain function. Even mTBI disturbs synaptic processes resulting in impaired network connectivity.^{41,42} Abnormal synaptic connectivity may reflect aberrant decreases in glutamatergic neurotransmission or overactivation of GABAergic inhibitory input.^{43,44} Levetiracetam (Keppra; UCB, Brussels, Belgium) is often used to control seizures in severe TBI patients.⁴⁵ Although the mechanisms of action are not fully understood they involve GABAergic activation and inhibition of presynaptic glutamate release.^{46,47} Cognition remains largely intact in patients on levetiracetam.⁴⁸ It is curious to speculate if levetiracetam may help balance long-term excitatory/inhibitory disturbances in neurotransmission after TBI. Zou et al⁴⁹ recently reported benefit from chronic treatment with levetiracetam after CCI in rats. Thus, one approach might be to use a potent NMDAR antagonist like memantine early after TBI, to block excitotoxicity and then transition to therapies that fine-tune glutamatergic activity during recovery such as levetiracetam. ►Fig. 1 provides an overview of excitotoxicity and its link to the neuronal death pathways along with emerging therapies.

Therapies Targeting Brain Edema

Brain edema has been a TBI target for decades. It is identified and continuously monitored in patients with severe TBI by imaging, clinical examination, and intracranial pressure (ICP) monitoring. Cerebral edema is caused by two main mechanisms—cellular (traditionally called *cytotoxic*) and vasogenic—resulting from a disturbance in the blood–brain barrier (BBB). There are no therapies in clinical practice designed to prevent edema, rather than simply to treat it once it has occurred. Treatments for brain edema are limited to the use of osmolar agents (mannitol, hypertonic saline), which aid in water removal, sedatives like barbiturates, which can lower the cerebral metabolic rate and reduce brain swelling (via a coupled reduction in cerebral blood volume), cerebrospinal fluid (CSF) drainage via a ventriculostomy, and craniectomy. These guidelines-based therapies are routinely used in severe TBI.^{50,51} However, they have toxicities. For example, increasing

serum sodium to > 170 mEq/L to manage refractory brain swelling after severe TBI caused an increased rate of acute renal failure, thrombocytopenia, and acute respiratory distress syndrome.⁵² Also, the decompressive craniectomy (DECRA) trial failed to show improved outcome in adults with severe TBI (although it decreased ICP).⁵³ Some have thus even begun to question the use of ICP monitoring in patients with severe TBI; this remains controversial.⁵⁴ A recent randomized controlled trial (RCT) by Chesnut et al⁵⁵ showed that outcomes after severe TBI did not differ between patients managed with ICP monitoring versus clinical exam/imaging—although the use of therapies for brain swelling was similar or greater in the patients treated based on the clinical exam/imaging. In contrast, other recent clinical studies have shown that even short periods of increased ICP unfavorably affect outcome,⁵⁶ and new preclinical work suggests that very modest levels of raised ICP (< 20 mm Hg) may be deleterious.⁵⁷ Brain edema might even contribute to secondary damage in mTBI if astrocyte swelling at the cellular level compromises astrocyte function.

Novel Pathways of Edema Formation

New understandings of the molecular underpinnings of brain edema are revealing new targets and therapies. Laird et al⁵⁸ outlined molecular events that could contribute to the development of brain edema after TBI (►Fig. 2). After TBI, neuronal necrosis can induce release of the danger signal high-mobility box protein 1 (HMGB-1). HMGB-1 binds to toll-like receptor-4 (TLR-4) on microglia and triggers interleukin-6– (IL-6–) mediated aquaporin-4 (AQP4) channel upregulation in astrocytes, mediating edema formation. AQP4 is a membrane channel that regulates water transport.⁵⁹ Consistent with this hypothesis, IL-6 and HMGB-1 are increased in human CSF after severe TBI.^{60,61} HMGB-1 is also linked to brain edema in TBI models.⁵⁸ In addition to binding to TLR4, HMGB-1 interacts with the receptor for advanced glycation end product (RAGE); the RAGE pathway may play a role in vasogenic edema/breakdown of BBB,⁶² while TLR4 may mediate cellular edema.⁵⁸ Cerebrospinal fluid levels of HMGB-1 correlate with unfavorable outcome after TBI.⁶¹ More discussion of this pathway is provided later in this review. Finally, Simard et al⁶³ reported that sulfonylurea receptor 1 (SUR1) contributes to the development of brain edema. SUR1 channels conduct monovalent cations, are upregulated after TBI, and function independent of Na^+ -K⁺ ATPase activity.⁶³

Emerging Therapies to Prevent Brain Edema

Effort has been directed at developing new therapies for brain edema in TBI. The anti-inflammatory drug glycyrrhizin inhibits HMGB-1 from binding to RAGE and prevents BBB breakdown and vasogenic edema.^{58,62} Similarly, Okuma et al⁶⁴ reported a reduction of edema in a rodent model using an anti-HMGB1 monoclonal antibody. Edema could also be reduced by inhibiting TLR4 with VGX-1027, which is in clinical trials for inflammatory diseases.⁵⁸ Other therapies targeting HMGB-1/TLR4 are discussed later. AQP4 is another edema target in TBI. Injection of small-interfering RNA targeting AQP4 reduced brain edema in rats⁶⁵ and selective AQP4

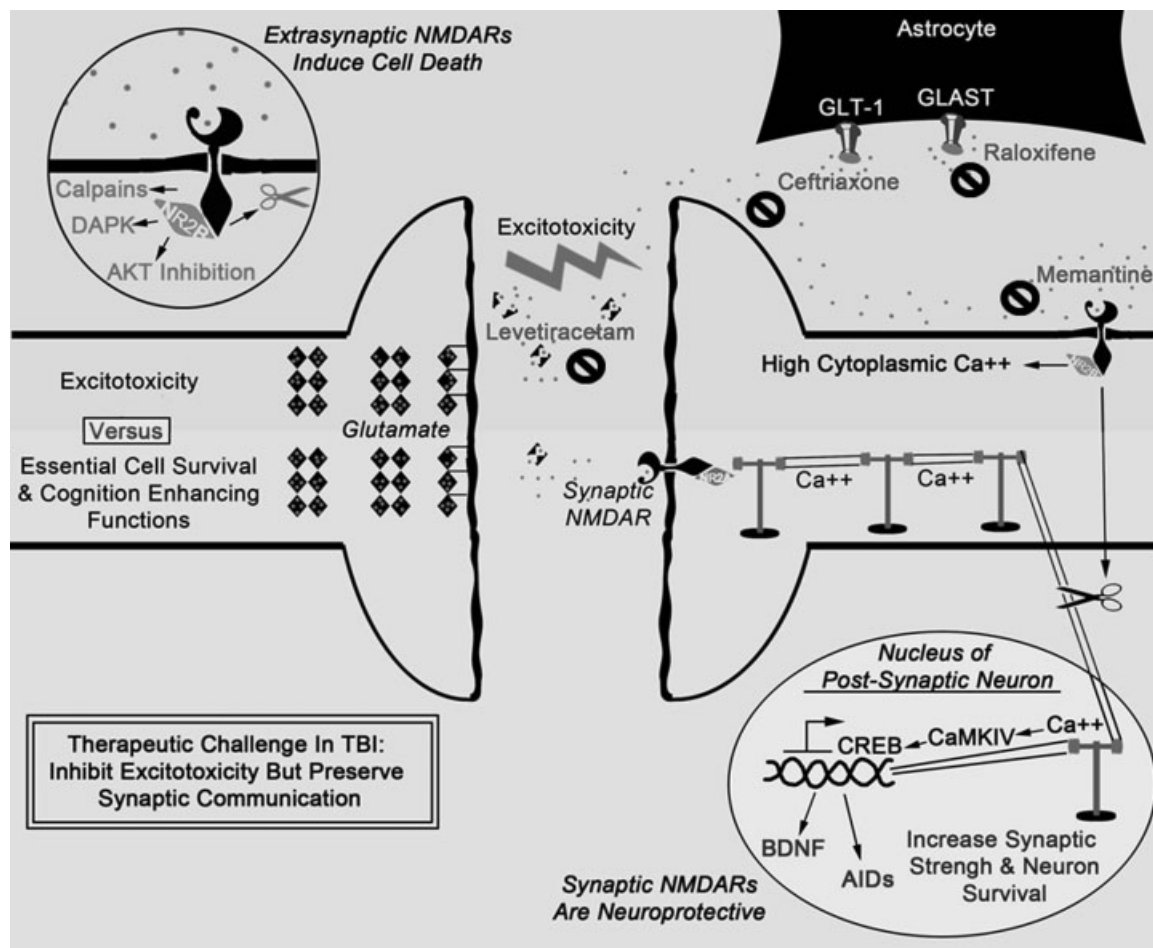


Fig. 1 Strategies to modulate traumatic brain injury- (TBI-) induced excitotoxicity while maintaining beneficial glutamatergic activation. Glutamate is an essential neurotransmitter in brain function. Normal release of glutamate from presynaptic neurons activates synaptic NMDARs (NR2A enriched) on postsynaptic neurons. Synaptic NMDAR activation promotes nuclear Ca^{2+} influx. Synaptic-to-nuclear Ca^{2+} communication transmits prosurvival signals (CaMKIV and CREB). The transcription factor CREB induces neuroprotective BDNF. Also, synaptic NMDAR activity stimulates expression of other protective AIDs. Traumatic brain injury alters Ca^{2+} biochemistry to favor toxic cytoplasmic signaling. Extracellular glutamate activates distal extrasynaptic NMDARs (NR2B enriched). Extrasynaptic NMDARs promote cell death (top-left). They oppose synaptic NMDAR/CREB prosurvival responses and activate calpains and DAPK, and inhibit AKT survival signaling. Drugs approved by the U.S. Food and Drug Administration targeting excitotoxicity include (identified by Ø): (1) Memantine— blocks extrasynaptic NMDAR, (2) ceftriaxone and raloxifene— increases expression of glutamate uptake transporters in astrocytes, and (3) levetiracetam—may inhibit high presynaptic glutamate release by modulating inhibitory GABAergic input to excitatory neurons. ALS, amyotrophic lateral sclerosis; CBF, cerebral blood flow; CTE, chronic traumatic encephalopathy; NMDAR, n-methyl-D-aspartate receptor; GluN2A/NR2A, glutamate N2A subunit; GluN2B/NR2B, glutamate N2B subunit; CREB, cAMP response element binding protein; GLT-1, glial glutamate transporter 1; GLAST, glutamate/aspartate transporter; AKT, protein kinase B; DAPK, death-associated protein kinase; BDNF, brain-derived neurotrophic factor; AID, activity-regulated inhibitor of death; CaMK, Ca^{2+} /calmodulin dependent protein kinase.

antagonists are in development (personal communication, Marc Pelletier). The SUR1 receptor blocker glibenclamide (Glyburide) is another promising therapy that reduces edema in TBI models.⁶⁶ It is in a phase II clinical trial (NCT-01132703) in TBI. These new agents give hope for improved management of brain edema. Studies in preclinical models are also needed to determine if reducing edema blunts secondary injury independent of ICP or reduced CBF, including studies in mTBI.

Mitochondrial Targeting Therapies and Oxidative Stress

Mitochondria have important functions ranging from generation of ATP to production of reactive oxygen species (ROS),

which are important in the regulation of life and death decisions in cells.⁶⁷ Dysfunctional mitochondria can also generate inflammatory and vasoactive mediators.⁶⁸ Timely elimination of dysfunctional mitochondria via macroautophagy is essential particularly in postmitotic cells such as neurons.⁶⁹ Mitochondrial dysfunction has been reported in experimental models and humans after TBI,^{70–73} and is a robust target because alterations in mitochondrial function persist days after injury.⁷⁴

Role of Oxidative Stress in Traumatic Brain Injury

Oxidative stress plays a key role after TBI.⁷⁵ It is classically described as a misbalance between the generation of free radicals and the body's ability to detoxify them.⁷⁶ However, this definition fails to describe the essential roles free radicals

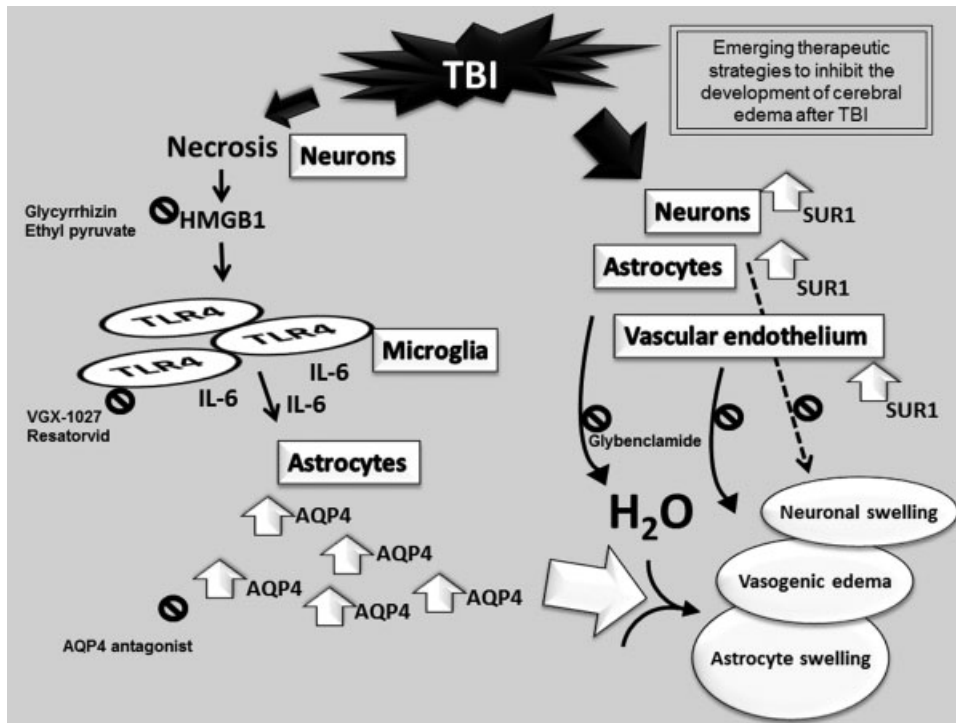


Fig. 2 Emerging therapies to inhibit the development of brain edema after traumatic brain injury (TBI). On the left, the novel molecular pathway of brain edema formation proposed by Laird et al.,⁵⁸ which hypothesizes that in areas of necrosis, HMGB1 release from neurons binds to TLR4 receptors on microglia and leads to elaboration of IL-6. IL-6 triggers upregulation of AQP4 water channels in astrocytes with water uptake across the blood–brain barrier and astrocyte swelling. On the right, a second pathway involves upregulation of the SUR1 channel on neurons, astrocytes, and cerebrovascular endothelium. This monovalent cation channel is normally absent but is upregulated after TBI. Emerging therapies targeting both pathways (identified by Ø). For the HMGB1-TLR4 cascade (left), potential therapies include (1) the HMGB1 binding agent glycyrrhizin or release inhibitor ethyl pyruvate, (2) inhibitors of TLR4 signaling (VGX-1027 or resatorvid), or (3) AQP4 antagonists. The prototype therapy targeting the SUR1 channel pathway (right) is glibenclamide. HMGB-1, high-mobility box protein 1; TLR4, toll-like receptor-4; IL-6, interleukin-6; AQP4, aquaporin-4; SUR1, sulfonyleurea receptor 1.

play in normal neuronal function, such as long-term potentiation.⁷⁷ A more contemporary definition, “disruption of redox signaling and control,” recognizes the compartmentalized nature of these events.⁷⁸ For a thorough evaluation of oxidative stress, a battery of studies, including (1) assessment of generation of free radicals; (2) quantification of oxidation products of lipids, proteins, and DNA; and (3) evaluation of radical scavenging capacity, should be performed. All of these components of oxidative stress occur after experimental and clinical TBI.^{79–83} Several sacrificial antioxidants and free radical scavengers have been explored in TBI, such as PEG-SOD, tirilazad, and edaravone.^{84–86} Although they have shown efficacy in TBI models, clinical trials failed to confirm efficacy.^{87,88} The limitations of general antioxidant strategies include narrow therapeutic window, inability to cross the BBB, and lack of targeting of the specific causes of oxidative stress. Without a targeted approach, the use of sacrificial antioxidants and free radical scavengers will fail.

Mitochondrial Failure after Traumatic Brain Injury

Mitochondria are a major intracellular source of ROS with production of superoxide and its dismutation product hydrogen peroxide.⁸⁹ Other sources of oxidative stress after TBI include NADPH oxidase, nitric oxide (NO) synthases, xanthine oxidase, and transition metals, which can be released by

hemorrhage.^{90,91} Underlying mechanisms of increased production of ROS by mitochondria include dysfunction of electron transport⁹² and impairment in Ca^{2+} buffering.⁹³ Mitochondria are also targets of ROS, which can promote MPTP opening, leading to apoptosis.⁹⁴ Mitochondrial DNA, which encodes elements for electron transport, is also a target for free radical damage.⁹⁵ Thus, oxidative stress can impair mitochondrial function, which in turn generates more oxidative stress in a vicious cycle.

Targeting Mitochondrial after Traumatic Brain Injury

Several strategies have been designed to combat mitochondrial dysfunction, including alternative fuels and MPTP inhibitors.^{96,97} Preliminary studies in adults describe the safety of cyclosporine, a MPTP inhibitor, when given after severe TBI.⁹⁸ These strategies target different components of mitochondrial dysfunction, but fail to localize into mitochondria. Recently small molecules have been discovered⁹⁹ that can selectively accumulate in mitochondria and bind targets in the organelle to exert their effects. Several strategies have been used, including (1) conjugation to lipophilic cations such as triphenylphosphonium that take advantage of negative membrane potential of mitochondria,⁹⁹ and (2) binding to a specific mitochondrial target such as cardiolipin (CL), a phospholipid exclusively found in the inner mitochondrial

membrane.⁶⁷ Both strategies are effective at delivering therapies into mitochondria. Compounds in the first category have not been tested in TBI. One of them, Mito-Q (a ubiquinone moiety linked to triphenyl-phosphonium), was used in recent human trials in Parkinson disease and hepatitis C.¹⁰⁰ Compounds in the second category include Szeto-Schiller (SS) peptides.¹⁰¹ Their uptake into mitochondria is thought to be independent of membrane potential, with a high affinity binding to inner membrane.¹⁰¹ They contain four alternating aromatic amino acids and some have antioxidant activity. One of these peptides, SS-31, protects mitochondria, accelerates ATP recovery, and reduces infarct size in the heart.¹⁰¹ Another class of compounds in the second category promising for TBI are the hemigramicidin-nitroxides (GS-nitroxide), inspired by the shared ancestry between mitochondria and bacteria, taking advantage of chemical moieties used in antibacterial agents (the antibiotic gramicidin S) with high affinity for the inner membrane.¹⁰² One GS-nitroxide, XJB-5-131, was shown to partition almost exclusively into neuronal mitochondria in vitro, penetrate the BBB, prevent TBI-induced CL oxidation and caspase activation, and improve lesion volume and neurocognitive outcome after TBI.⁷⁰ This is an exciting targeted strategy for TBI.

In summary, there is promising experimental success in application of mitochondria-targeted redox regulators in treatment of TBI, and these approaches deserve significant future efforts.

Therapies Targeting Neuroinflammation

Evidence suggests the inflammatory response, including cytokines, chemokines, microglial activation, and recruitment of circulating leukocytes, mediates secondary injury and/or repair after TBI. Traumatic brain injury causes the release of endogenous danger signals (i.e., extracellular ATP and HMGB-1),^{58,61} which bind to pattern recognition receptors such as TLR4 on neurons and glia to activate the immune response. Activated microglia undergo a phenotypic shift from an anti-inflammatory (M2) state to a proinflammatory and procytotoxic (M1) state. M1 microglia proliferate and migrate to the injury site and (1) form a barrier between damaged and healthy tissue,¹⁰³ (2) increase expression of proinflammatory cytokines such as TNF α ¹⁰⁴ and IL-1 β ,¹⁰⁵ and (3) release ROS and reactive nitrogen species. Chronic microglial activation develops and may mediate chronic traumatic encephalopathy (CTE) and neurodegenerative diseases.¹⁰⁶

Dual Role of Inflammation in TBI

Studies of the role of TNF α after TBI reveal the dual effects of inflammation in secondary injury and repair. Scherbel et al¹⁰⁷ using a TNF KO mouse reported evidence of early neuroprotection in the KO at 48 hour; however, TNF KO mice had persistent motor deficits and more tissue loss at 4 weeks compared with wild-type. Balancing neurotoxicity with repair must be considered for therapies that modulate inflammation. Preclinical work with thalidomide analogs (TNF α synthesis inhibitor) and etanercept (fusion protein that binds to and inhibits TNF α) have shown benefit early after TBI.^{108,109}

HMGB-1/TLR4 Pathway Inhibition

HMGB1/TLR4 pathway inhibitors were previously discussed. They also block secondary injury due to immune activation. Ethyl pyruvate, an inhibitor of HMGB1 secretion, and resatorvid, a small molecule inhibitor of TLR4, improved outcome and reduced levels of TNF α and IL-1 β in rodent TBI models.^{110,111} These drugs have not yet been evaluated in clinical trials for TBI.

Other Anti-Inflammatory Agents

Minocycline, a lipophilic tetracycline antibiotic with several proposed mechanisms of action, including inhibition of microglial activation, reduces IL-1 β production, lesion volume, and functional deficits in TBI models.¹¹² A phase 1 clinical trial of minocycline in TBI is recruiting patients. IL-1 β antagonism via intraventricular injection of anti-IL-1 β antibody¹¹³ and transgenic overexpression of IL-1 receptor antagonist (IL-1ra)¹¹⁴ reduced lesion volume and improved outcomes in TBI models. A recent phase 2 trial in adults with severe TBI¹¹⁵ randomized 20 patients to receive 100-mg recombinant human IL-1ra (Anakinra) for 5 days, an FDA-approved dose for rheumatoid arthritis. Adverse events did not differ in treatment and control groups. Cerebral microdialysis showed increased levels of IL-1ra with treatment and a shift in the cytokine/chemokine profile. HMG-CoA reductase inhibitors (statins) have several proposed mechanisms of action after TBI (antiapoptotic, antioxidant, increase CBF, and neurogenesis); however, the primary mechanism is likely anti-inflammatory. Two clinical studies of statins have been conducted—a RCT of rosuvastatin in 20 adults with TBI that showed improved memory with treatment,¹¹⁶ and a retrospective study showing a 76% relative risk reduction for mortality in patients treated with statins before injury.¹¹⁷ Larger RCTs are needed.

Promoting Inflammation-Mediated Regeneration

Another approach is to promote shifting microglia from the M1 to the M2 phenotype. Currently under investigation for treating multiple sclerosis,¹¹⁸ therapies such as glatiramer acetate, interferon- β , or dimethyl fumarate may promote the beneficial aspects of neuroinflammation—neurogenesis and repair—while reducing cytotoxic mediators. A similar approach is being adapted from spinal cord injury research with the use of “pro-inflammatory” therapy: G-CSF alone¹¹⁹ or in combination with mesenchymal stem cells.¹²⁰ These therapies may also be useful late in the course of the disease to mitigate CTE.¹²¹

Therapies Targeting Traumatic Axonal Injury

Therapies targeting traumatic axonal injury (TAI) is a prominent feature of TBI and represents a vital target across the spectrum of injury severity. In classic studies, Povlishock^{122,123} showed that TAI is a fundamental component of secondary injury after TBI and thus a key therapeutic target. Progression of TAI involves TBI-induced dysregulation of Na⁺ channels, in turn causing increased Ca²⁺ influx into axons,

calpain activation with loss of microtubules, neurofilament impaction with impaired axoplasmic transport,¹²⁴ and mitochondrial failure with permeability transition pore opening and oxidative stress.¹²⁵ The aforementioned novel strategies targeting mitochondrial failure may be particularly efficacious in TAI. Smith et al¹²⁴ recently identified categories of therapies for TAI based on work in TBI models: (1) cytoskeleton stabilization, (2) ion homeostasis, (3) protease inhibition, (4) mitochondrial protection, (5) mild hypothermia, and (6) other therapies. We will review and update those categories (→ Fig. 3).

Cytoskeleton Stabilization

Loss of microtubule function devastates axonal transport. Unfortunately, data are limited on approaches to block this mechanism. The chemotherapeutic drug Taxol can inhibit chemical depolymerization of microtubules during stretch.¹²⁶ Taxol has not been tested in TBI in vivo, but recently produced axonal preservation after spinal cord injury in rats.¹²⁷

Ion Homeostasis

There has also been limited work targeting Ca^{2+} accumulation linked to TAI. Rather, focus has been on downstream signaling cascades such as calpain activation. Therapies targeting major breaches in membrane disruption such as Kollidon VA64 show promise in TBI models.¹²⁸

Protease Inhibition

Beneficial effects of calpain inhibition on TAI have been shown in rodent TBI models for over a decade, including use of MDL28170, AK295, and SJA-6017.^{124,129} However, lack of brain bioavailability and target specificity have limited the development of calpain inhibitors. Recent studies have suggested that citicoline suppresses calpain activation after TBI. However, the failed COBRIT clinical trial with citicoline argues against this agent.¹³⁰ Given the evidence supporting a role for calpain in TAI, it is disappointing that additional calpain inhibitors are not available.

Mitochondrial Protection

Mitochondrial failure may worsen Ca^{2+} overload and exacerbate TAI. Preclinical work¹²⁵ has shown benefit from cyclosporine A (CsA) on TAI. Inhibition of MPTP opening is suggested as the mechanism for this effect. However, clinical studies with CsA have been equivocal.³ The aforementioned GS-nitroxides⁷⁰ or N-acetyl cysteine (NAC) amide¹³¹ are logical candidates to study.

Hypothermia

Preclinical work shows that mild hypothermia can attenuate TAI.¹²⁹ Sadly clinical trials in TBI have failed.¹³² Surprisingly, hypothermia failed to attenuate the increase in CSF levels of myelin basic protein after severe TBI in children.¹³³ Mild hypothermia was recently shown to markedly attenuate TAI

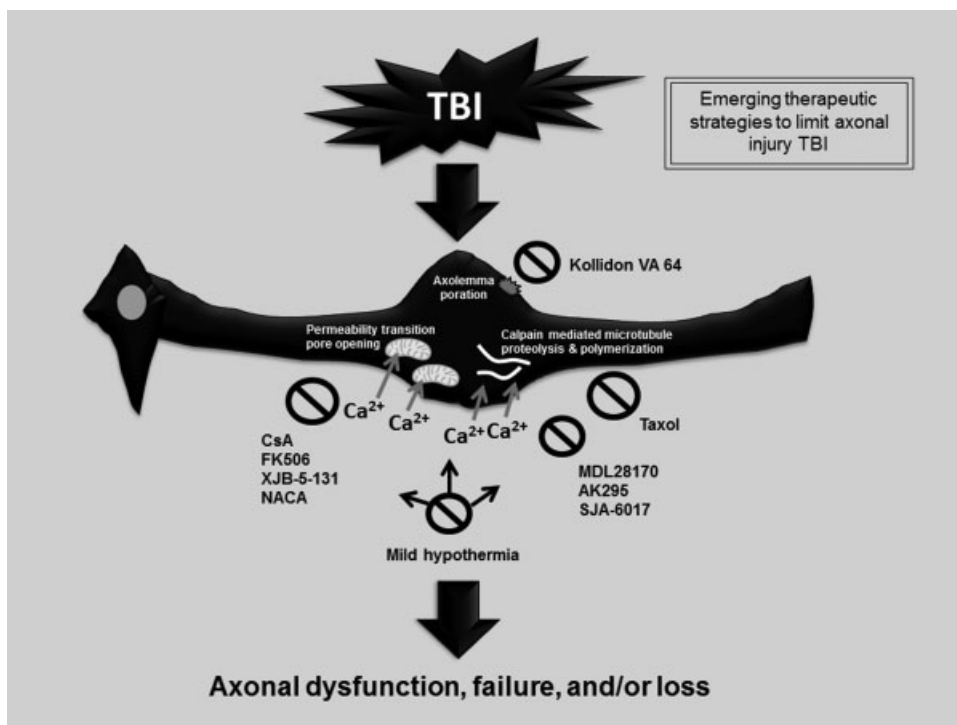


Fig. 3 Emerging therapies to limit traumatic axonal injury (TAI) after traumatic brain injury (TBI).¹²⁴ Cellular and molecular events in the TAI cascade include Ca^{2+} accumulation with calpain activation and resultant microtubule proteolysis, MPTP opening, and oxidative stress. Direct membrane poration can also mediate injury. Emerging therapies (identified by Ø) include calpain antagonists (MDL28170, AK295, SJA-6017), and taxol, which may prevent microtubule polymerization. Therapies targeting mitochondria include CsA that blocks MPTP, FK506 that may target calcineurin induced translocation of BAD, and XJB-5-131 and NACA that target oxidative stress in mitochondria. Kollidon VA64 may directly reseal membranes. Mild hypothermia may reduce TAI by multiple mechanisms. CsA, Cyclosporin A; NACA, N-acetyl cysteine amide.

after repetitive mTBI in rats.¹³⁴ Mild hypothermia deserves exploration in mTBI.

Other

In addition to CsA, the calcineurin inhibitor FK506 has been shown to reduce TAI, particularly in unmyelinated axons.¹³⁵ It is unclear whether this is independent of effects on mitochondria.¹²⁴ Therapies targeting oxidative damage have also been suggested to reduce TAI¹³⁴—these were discussed previously.

Therapies Targeting Cerebral Blood Flow Dysregulation and Ischemia

After TBI, CBF dysregulation develops and may contribute to secondary damage. In mTBI, vascular dysregulation could mediate vulnerability to a second hit.¹³⁶ Cerebral blood flow is often reduced early after severe TBI.¹³⁷ This may result from a coupled reduction in brain activity, but can be pathologic during excitation. Cerebrovascular resistance (CVR) is controlled at the macro- and microvascular level. At the microvascular level, it is coupled to brain metabolic activity. Recently, Hall et al¹³⁸ showed that pericytes positioned around brain capillaries mediate dilation that produces a much greater change in CBF (19% vs. 3%) than dilation of arteries alone—implicating pericyte-mediated dilation as the major contributor to changes in CBF. Key metabolites identified included the vasodilators nitric oxide (NO) and prostaglandin-E₂ (PGE₂) and the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE). They implicated the relative production of these metabolites in regulating pericyte dilation and thus CBF. The potent vasoconstrictor endothelin-1 (ET-1) may also contribute to CBF dysregulation after TBI.

Modulating Nitric Oxide

Nitric oxide is a potent vasodilator and may be useful to treat ischemia after TBI. Conversely, NO at high levels can be converted to peroxynitrite, which can worsen damage. This paradox has yielded interventions that augment NO delivery or block NO production after TBI. The primary clinical strategy to increase NO delivery is with inhaled NO. Inhaled NO is FDA-approved for neonatal respiratory failure. Studies suggest that inhaled NO improves collateral circulation and outcomes in TBI models.¹³⁹ However, in newborn piglets, the NO donor sodium nitroprusside did not prevent impaired autoregulation during hypotension after TBI, implying that NO augmentation may only be beneficial in normotensive states.¹⁴⁰ This is important, given that NO augmentation can produce hypotension that may mitigate benefit. Whether inhaled NO is a viable approach remains to be determined. Nitric oxide synthase inhibitors are also being explored in TBI. A phase II placebo controlled trial with the NO synthase inhibitor VAS 203 was just completed in 32 adults with TBI (NOSTRA; NCT 02012582). It suggested improved outcomes, but renal injury was a concern. It is unknown if NO augmentation or inhibition is preferred. A targeted approach to NO delivery to maximize microvascular effects coupled with selective inhibition of inducible NOS (iNOS) to prevent nitro-

sative stress might be considered. However, sustained inhibition of iNOS may be deleterious given that iNOS KO mice exhibit marked impairments in cognitive outcome.¹⁴¹

Modulating ET-1

In TBI, ET-1 levels in CSF are increased and linked to unfavorable outcomes.¹⁴² However, in a phase 2b trial, the ET receptor A antagonist clazosentan failed to improve outcomes after subarachnoid hemorrhage (SAH).¹⁴³ New ET-1 antagonists remain to be tested in TBI.

Statins

HMG-CoA reductase inhibitors (statins) are used for cholesterol reduction. As discussed, they have anti-inflammatory effects, but also upregulate eNOS and increase NO production, leading to improved capillary patency.¹⁴⁴ Clinical trials with statins suggest a potential benefit on outcome in TBI, although the role of effects on CBF is unclear.¹⁴⁵ In stroke, an RCT of lovastatin is in phase 2 trials (NeuSTART, NCT01976936).¹⁴⁶ The optimal choice of statin and dosing for BBB penetration also remain unclear. Results from larger RCTs in TBI are needed to define the utility of these agents in TBI.

Cytochrome P450 Metabolites

Cytochrome P450 produces two classes of arachidonic acid metabolites with opposing microvascular effects. Hydroxylation produces 20-HETE, a potent vasoconstrictor, while epoxidation produces epoxyeicosatrienoic acids (EETs), which are vasodilatory. Both 20-HETE and EETs are autoregulatory mediators. Inhibition of 20-HETE formation by NO is an essential pathway of PGE₂-mediated pericyte dilation.¹³⁸ Inhibition of 20-HETE formation or prevention of EET metabolism can reduce lesion volume in stroke and SAH models.^{147,148} 20-HETE is involved in reduced CBF in cortical spreading depression¹⁴⁹—a secondary injury mechanism implicated in TBI.¹⁵⁰ Thus, cytochrome P450 arachidonic acid metabolites affect CBF after TBI. It remains to be determined whether targeting these pathways can improve outcome after TBI.

Enhancing Oxygen Delivery

Another approach to reduce ischemic damage is to improve oxygen delivery despite CBF reductions—such as with perfluorocarbon-enhanced oxygen delivery. A safety/efficacy RCT is evaluating the perfluorocarbon Oxycyte (STOP-TBI; NCT00908063). Mild hypothermia has also been shown to reduce CBF dysregulation after TBI.¹⁵¹ This includes benefit injury in repetitive mTBI.¹³⁴

In summary, an emerging area TBI is mitigating microvascular dysregulation. These approaches and others deserve further investigation in TBI, including testing in mTBI.

Cognitive Enhancement

Many rehabilitation strategies have been used to enhance cognitive function after TBI. Some of the most effective preclinical rehabilitative strategies in TBI have been neurostimulant pharmacotherapies, and as discussed below,

successful clinical translation of this approach in an RCT was recently achieved.² Thus, we will focus on these agents as emerging TBI therapies.

Catecholamine Agonists

Catecholamine agonists promote functional recovery after TBI. This was confirmed in weight drop cortical contusion or ablation models in rats and/or cats.^{151–154} Because norepinephrine antagonists block or reinstate deficits, the noradrenergic system has been implicated. However, clinical^{155,156} and experimental^{157,158} research shows that the dopamine (DA) system is also involved in both injury and rehabilitative processes. Methylphenidate, a psychostimulant and DA transporter inhibitor, exhibits pharmacological properties similar to amphetamine, but without undesirable sympathomimetic effects. In a study assessing motor function after sensorimotor cortex injury in rats, a single dose of methylphenidate followed by symptom relevant experience (beam walking) enhanced beam-walk ability.¹⁵⁴ This work supports the importance of an interaction between pharmacotherapy and symptom-relevant experience (rehabilitation) in promoting functional recovery after TBI. Moreover, daily treatment with methylphenidate beginning as late as 24 hours after CCI in rats revealed less spatial memory deficits versus controls.¹⁵⁹ Wagner et al¹⁶⁰ has showed that methylphenidate exhibits some restorative capacity for striatal DA neurotransmission after experimental TBI; their additional work suggests potential sex differences in methylphenidate treatment effects and dosing for male versus female rats.¹⁶¹

Amantadine—A Translational Success

With success in preclinical studies as well as phase 2 and phase 3 clinical trials, amantadine (Symmetrel; Endo Pharmaceuticals, Malvern, PA) is a promising drug for TBI rehabilitation.^{2,162} Daily treatment with amantadine (for 20 d) after CCI in rats revealed improvements in spatial memory performance deficits versus saline-treated counterparts.¹⁶³ Higher doses also benefit cognition after fluid percussion.¹⁶⁴ Clinically, amantadine accelerates the rate of functional recovery in vegetative or minimally conscious patients during the subacute phase after TBI.² It exerts its effects by increasing extracellular DA by blocking reuptake and by facilitating synthesis.^{165,166} In addition to its presynaptic actions, amantadine increases the density of postsynaptic DA receptors¹⁶⁶ or alters their conformation, which may be clinically important.¹⁶⁷ Because the mechanism of action of amantadine differs from other DA-releasing drugs,¹⁶⁸ it is likely that the dopaminergic effects of amantadine are a combination of presynaptic and postsynaptic effects. It also blunts NMDAR activation.

Bromocriptine

Delayed or chronic treatment with the D2 receptor agonist bromocriptine also improves the acquisition of spatial and working memory in rats after CCI.¹⁶⁹ Bromocriptine (5 mg/kg) has been shown to increase extracellular DA levels in rats,¹⁷⁰ suggesting that enhanced presynaptic DA neurotransmission may have mediated the benefit at this dose. Bromocriptine also

attenuated lipid peroxidation, suggesting antioxidant effects.¹⁶⁹ Further support for dopaminergic activity in restoring functional recovery after TBI comes from a preclinical study showing that selegiline (L-deprenyl), which enhances the action of DA by inhibiting its main catabolic enzyme in brain, monoamine oxidase-B, improved cognitive outcome when given daily for 7 days after fluid percussion injury.¹⁷¹ Clinical studies showing benefits of DA augmentation after TBI also exist.^{172,173}

Thus enhancing catecholamine neurotransmission during the chronic postinjury phase may be a useful adjunct in ameliorating the neurobehavioral sequelae of TBI in humans. Additional studies are warranted.

Augmenting Endogenous Neuroprotectants

Evolution has provided several naturally occurring neuroprotective mechanisms. Perhaps the “low-hanging fruit” for increasing therapeutic options for TBI patients resides in augmenting the mechanisms that allowed the brain to evolve in the first place.

Adenosine as an Archetype for Endogenous Neuroprotection

Adenosine is released by tissue injury via several pathways. Breakdown of ATP is one source of adenosine in the injured brain (the traditional route). Recently, an alternative 2',3' cAMP pathway was discovered, and involves production of adenosine from mRNA breakdown via 2',3'cAMP. This latter pathway appears to play a major role after TBI.¹⁷⁴ Adenosine acts on cell surface receptors (A1, A2A, A2B, and A3) and engages signal transduction that is neuroprotective in TBI.¹⁷⁵ Activation of A1 receptors attenuates post-TBI excitotoxicity. Mice null for A1 receptors suffer lethal status epilepticus after TBI,¹⁷⁶ and variants in the A1 receptor genes associate with posttraumatic seizures in TBI patients.¹⁷⁷ Moreover, A1 receptor KO mice exhibit enhanced microglial proliferation after TBI.¹⁷⁸ However, systemic effects of A1 agonists (bradycardia, hypotension) limit their use in TBI. A more effective strategy might be to enhance adenosine levels in the brain or increase A1 receptor numbers and/or signaling. One approach would be to upregulate enzymes that produce adenosine. For example, isoflurane increases activity of the adenosine-forming enzyme ecto-5'-nucleotidase (CD73) by stimulating release of microparticles,¹⁷⁹ which may contribute to its neuroprotection.¹⁸⁰ Another approach would be to administer chronically and prophylactically an A1 receptor blocker with a short half-life. This would upregulate A1 receptor numbers and signaling in the brain, yet post-TBI the antagonist would dissipate rapidly, leaving enhanced adenosine signaling at the time of greatest need. Improved outcomes are seen in TBI patients with caffeine (a short half-life adenosine receptor antagonist) in their CSF at the time of injury.¹⁸¹ Finally, adenosine kinase (ADK) is a key enzyme in the breakdown of adenosine. Adenosine kinase increases markedly in the astrocyte scar and limits adenosine availability chronically after TBI.¹⁸² Given the anticonvulsant effects of A1 receptors, blocking ADK or using other strategies to

overexpress adenosine may represent a therapy for posttraumatic seizures. Huber et al¹⁸³ used grafts of adenosine-releasing fibroblasts to suppress seizures in rats. A1 receptor gene polymorphisms are strongly associated with posttraumatic seizures.¹⁷⁷ Thus, it may be possible to use a personalized medicine approach to define patients who might benefit from adenosine augmentation therapy.

Other Endogenous Neuroprotectants

There are many other endogenous neuroprotectants emerging as therapies for TBI. Augmentation of trophic factors such as BDNF, or immediate early gene products such as HSP, could improve outcome after TBI.¹⁸⁴ It was recently shown that remote preconditioning using tourniquet inflation/deflation may mediate benefit in ischemia/reperfusion via local elaboration of nitrite—which is converted to NO in regions of tissue hypoxia.¹⁸⁵ Systemic nitrite therapy might yield similar effects.¹⁸⁶ An endogenous neuroprotectant receiving attention in Parkinson disease is uric acid, which has antioxidant effects.¹⁸⁷ Finally, regulators of cold stress such as RNA-binding motif 3 (RBM3) stabilize mRNAs and may underlie benefit in hypothermia.¹⁸⁸ New drugs are targeting RBMs.¹⁸⁹

Cellular Therapies

Therapies designed to replenish cells lost after TBI may help improve outcome. Methodologies include supplementation of exogenous stem cells or therapies that enhance endogenous neurogenesis in the adult brain. Both approaches have promise based on studies in TBI models. New approaches are being used to enhance the regenerative capacities of these cellular therapies in hopes of developing interventions that can ultimately promote recovery in patients.

Administration of Exogenous Cells

Traumatic brain injury can be associated with loss of neurons and other cells in multiple brain regions. Administration of exogenous bone marrow stromal cells in rats, via intracranial,¹⁹⁰ intra-arterial,¹⁹¹ or intravenous delivery,¹⁹² results in a portion of transplanted cells migrating into the brain parenchyma, and is associated with improved motor function after TBI.^{190,193} Survival of stromal cells is associated with increased production of BDNF and nerve growth factor.¹⁹² In other studies, transplantation of neural stem cells^{194,195} and the coadministration of neural stem cells and olfactory ensheathing cells similarly improve motor performance after TBI.¹⁹⁶ However, it is unclear whether the regenerative capacity of transplantation is dependent upon the incorporation of the exogenous cells, the production of soluble growth permissive factors, or their combination. Two studies provide evidence for complex dynamics between the contributions of exogenous cell survival and the release of soluble factors that promote regeneration after TBI. Intracranial administration of human bone marrow stromal cells in a collagen scaffold matrix enhances the survival of the stromal cells in the cortex and improves motor function compared with administration of stromal cells alone.⁵² Coadministration of the collagen scaffold also enhanced corticospinal tract

sprouting in the denervated spinal cord,¹⁹⁷ suggesting contributions from both the surviving stromal cells and soluble factors that promote regeneration after TBI. Tajiri et al¹⁹⁸ studied soluble factors released from transplanted human adipose-derived stem cells that may play a role in recovery after TBI. Delivery of either the adipose-derived stem cells or conditioned media improved outcome in rats after TBI. However, knockdown of two long noncoding RNAs, important for cellular differentiation, blunted the recovery. These data indicate that regeneration can be enhanced with soluble factors in the absence of transplanted cells. They reveal the potential of transplantation approaches and highlight the complex dynamics between stem cells and growth permissive factors in promoting recovery.

Enhancing the Generation and Survival of Newborn Cells

Therapies that enhance the generation of newborn neurons are also attractive after TBI. Enhancement of neurogenesis after TBI with growth factors, neuroprotective agents, and hypothermia promotes cellular proliferation and increases the generation of newborn neurons in neurogenic regions of the injured brain.^{199–202} These studies have also shown that an enhancement of posttraumatic neurogenesis in the weeks after TBI is associated with improved neurobehavioral performance. Hippocampal immature neurons are particularly sensitive to brain injury in the days postinjury.²⁰² Promoting survival of neural stem cells and immature neurons is a promising target to improve outcome.²⁰³ Blaya et al²⁰⁴ evaluated the efficacy of the neuroprotective agent P730-A20 to promote the survival of immature neurons, as this drug blocks apoptosis in immature neurons. Treatment with P730-A20 improved immature neuron density, increased the number of newly generated neurons, and improved cognitive performance. This highlights the promise of therapies promoting newborn neuron survival and incorporation into the injured brain.

Clinical Trials of Cellular Therapy

There are several clinical trials exploring cellular therapy in TBI. Recently a study addressing “Safety of Autologous Stem Cell Treatment for TBI in Children” (NCT00254722) was completed. The objective of that phase 1 study was to determine if bone marrow precursor cell harvest and autologous transplantation (within 36 h of injury and by intravenous route) is safe in children after TBI. The study was completed; a phase II trial is recruiting (NCT01851083). There is also an open label study of “Autologous Bone Marrow Mononuclear Cells in TBI” (NCT0202810), in which bone marrow-derived mononuclear cells are given intrathecally. That study is also recruiting.

Combination Therapy

Combination therapy is attractive to overcome translational challenges. Incomplete understanding of dose-response relationships and poor central nervous system (CNS) penetration of therapies are factors widely acknowledged to contribute to

failed clinical trials.^{205–207} The heterogeneity of TBI and its complex pathophysiology suggest that it is unlikely that any single agent can address all of the secondary injury mechanisms.²⁰⁵ The success of combination therapies that enhance drug exposure or have complementary mechanisms of action in cancer and human immunodeficiency virus has further increased enthusiasm for this approach in TBI research.

Emerging Combination Therapies

In 2008, the NIH convened a workshop on multidrug combinations for TBI. The recommendation was to combine therapies with complementary targets and effects rather than focus on a single target with multiple therapies.²⁰⁷ Several combination therapies meeting this definition are being investigated. One of the most promising combines the anti-inflammatory agent minocycline and the glutathione precursor NAC. Effects of minocycline were discussed previously. N-acetyl cysteine is a precursor for synthesis of the antioxidant glutathione, impacts glutamatergic transmission, and despite poor CNS penetration improved outcomes in some TBI models and in blast-induced mTBI in humans.^{208–210} When given together, benefits of the combination exceed that of the single agents studied in CCI and mTBI models.^{211,212} N-acetyl cysteine is used in another combination therapy designed to improve drug exposure. Our group is testing coadministration of the FDA-approved organic acid transporter and multidrug resistance-associated protein inhibitor, probenecid, with NAC in preclinical and phase I pediatric studies (NCT01322009). The aim is to overcome membrane barriers, such as the BBB, to synergistically improve NAC bioavailability and antioxidant reserves after TBI. Preclinical pharmacokinetic (PK) data show that probenecid increases NAC brain penetration in juvenile rats two- to threefold as early as 1 hour after injury.²¹³ Outcome studies are underway. Other combinations such as progesterone plus vitamin D are in early stages of preclinical investigation.²¹⁴

Addressing Unique Challenges of Combination Therapy

Interactions between therapies may alter PK (dose-concentration relationships) or pharmacodynamic (concentration-effect relationships) properties of either therapy. They may be additive, synergistic, or antagonistic. Thus, studies using full-factorial designs at multiple dosing levels are ideal.²⁰⁷ These data are used to identify combinations and sequences of therapies that achieve greater efficacy and lower toxicity than either therapy alone. Specific statistical approaches to identify synergism are advocated.²¹⁵ Coadministered therapies should also be evaluated for physiochemical incompatibilities to ensure systemic bioavailability. As with single drugs, it is imperative to measure brain concentrations of therapies used in combination to optimize their potential for success.

Therapies Targeting TBI Resuscitation in Polytrauma

Traumatic brain injury is often accompanied by secondary insults (hypotension, hemorrhage, hypoxemia) that worsen

outcome.²¹⁶ However, given their complexity, clinical studies commonly exclude these patients, and few animal models have been developed to investigate therapies. Optimal resuscitation of the TBI patient with polytrauma continues to present unique challenges and remains understudied.

Resuscitation Fluids

The mainstay of resuscitation involves fluids: crystalloids, colloids, and/or blood products. Crystalloids are the initial therapy; however, large volumes are often needed, which can exacerbate brain edema.²¹⁷ Colloids, given their improved ability at maintaining intravascular volumes, are attractive; however, in the SAFE study, TBI patients resuscitated with albumin had raised ICPs and greater mortality compared with saline-treated patients.²¹⁸ Traumatic brain injury-induced BBB permeability may have allowed extravasation of albumin into brain—potentiating rebound brain edema.²¹⁹ Thus, small molecule colloids may be problematic early after TBI. Blood products are not available for prehospital use.

Emerging Resuscitation Agents

Given the risk of exacerbation of brain edema with current resuscitation fluids, new therapies are being investigated, including novel, ultra-small-volume resuscitation agents. Polynitroxylated pegylated hemoglobin (PNPH) is one agent that may represent an out-of-hospital bridge to transfusion. Polynitroxylated pegylated hemoglobin is a bovine-based hemoglobin that, in an effort to eliminate toxicity of cell-free hemoglobin, is covalently bonded with antioxidant nitroxide moieties and polyethylene glycol side chains. Polynitroxylated pegylated hemoglobin is being developed as a small-volume resuscitation solution. In a model of TBI plus hemorrhage, it dramatically reduced resuscitation fluid requirements compared with crystalloid.^{220,221} It also reduced ICP, brain edema, and neuronal death.^{220,221} Unlike conventional free hemoglobins, it has surprising *in vitro* neuroprotective effects.²²¹ It is in preclinical development. Traditional resuscitation approaches focus on improving tissue perfusion by increasing circulating blood volume. An alternative might entail modification of microcirculatory blood flow. Drag-reducing polymers (DRPs) at nM levels markedly reduce the resistance of microvascular flow, improving tissue perfusion.^{222,223} Drag-reducing polymers, such as long-chain polyethylene glycol (kDa > 10⁶), improve perfusion and reduce mortality in models of hemorrhagic shock.²²⁴ Drag-reducing polymers thus could maintain brain perfusion despite using a volume-limited resuscitation.

Conventional Resuscitation Plus Antiedema Therapies

Although volume-limiting resuscitation strategies hold promise in TBI, treatment with the aforementioned novel drugs targeting brain edema (Kollidon VA64, glibenclamide, AQP4 antagonists) could reduce the deleterious effects of resuscitation fluids on edema after TBI. Preclinical studies of these drugs are needed in models of TBI plus hemorrhage/polytrauma.

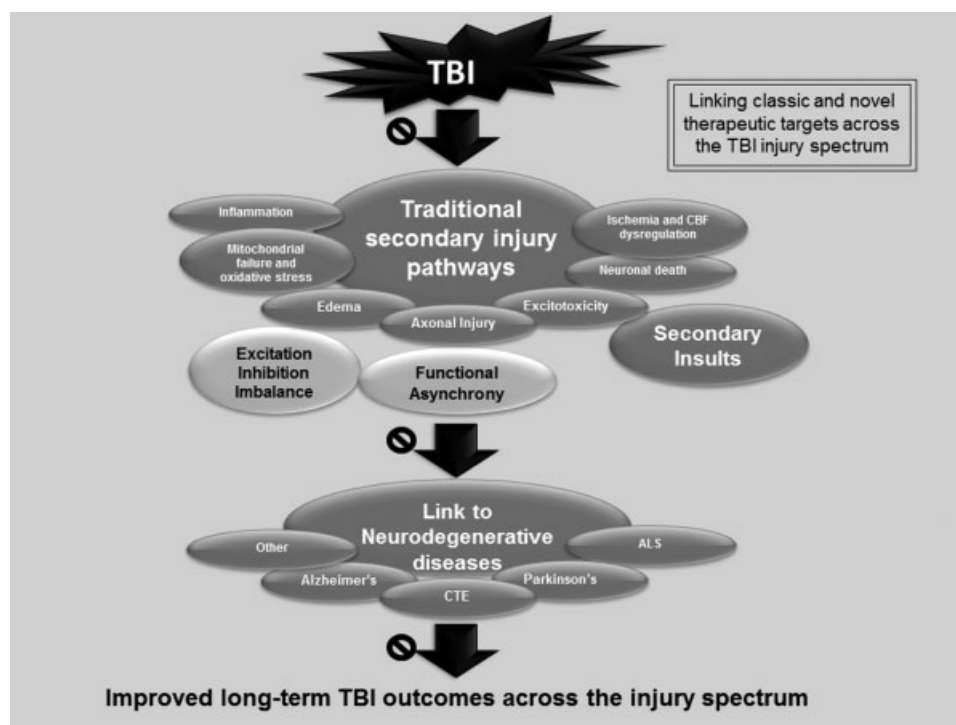


Fig. 4 A contemporary view of therapeutic targets across the spectrum of traumatic brain injury (TBI) from mild to severe includes classical secondary injury pathways along with novel mechanisms such as excitation inhibition imbalance and functional asynchrony that have come to the forefront in mild TBI. Therapies to embellish rehabilitation-based strategies to break the link between TBI and chronic neurodegenerative diseases such as chronic traumatic encephalopathy (CTE) are also needed. Therapies are identified by Ø. ALS, amyotrophic lateral sclerosis.

Other Therapies

Many more emerging therapies are on the horizon, including the use of transcranial low level laser,²²⁵ nutraceuticals,^{226,227} lithium,²²⁸ modulating cell cycle²²⁹ and targeting microhemorrhage,²³⁰ among many others. This brief list illustrates the level of creativity in our dynamic research field.

Conclusion

This review is far from comprehensive. However, given our goal to address mechanism-based emerging therapies across the injury-severity spectrum, and from the field to rehabilitation, we chose to provide a “survey” across key mechanisms. There are many other promising agents worthy of investigation. We also believe that the recent surge in interest in mTBI may identify new targets in severe TBI—particularly given the fact that in patients with severe TBI, brain regions outside of areas of major disruptions are likely to be plagued by the pathomechanisms seen in mTBI. Thus, new investigations into therapies for mTBI may provide new opportunities for treatment of severe TBI. For example, emerging mechanisms in mTBI, such as disturbances in the balance between excitatory and inhibitory pathways or disturbances in synchronization,^{42,231} could be important in severe TBI—layered upon the classical injury paradigms (→Fig. 4). Finally, to complement rehabilitation and cognitive enhancing therapies currently used chronically after TBI, new approaches to break the link between TBI and chronic

neurodegenerative diseases including CTE are needed (→Fig. 4). The current golden age of TBI research thus represents a special opportunity for the development of breakthroughs in the field.

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