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Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies

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Abstract

Traumatic brain injury (TBI) causes secondary biochemical changes that contribute to subsequent tissue damage and associated neuronal cell death. Neuroprotective treatments that limit secondary tissue loss and/or improve behavioral outcome have been well established in multiple animal models of TBI. However, translation of such neuroprotective strategies to human injury have been disappointing, with more than thirty controlled clinical trials having failed. Both conceptual issues and methodological differences between preclinical and clinical injury have undoubtedly contributed to these translational difficulties. More recently, changes in experimental approach, as well as altered clinical trial methodologies, have raised cautious optimism regarding outcomes of future clinical trials. Here, we critically review developing experimental neuroprotective strategies that show promise and propose criteria for improving the probability of successful clinical translation.

Introduction

Traumatic brain injury (TBI) is a major cause of mortality and morbidity, particularly at the two ends of the age spectrum, with large direct and indirect costs to society. In the United States (US) it has been estimated that more than 1.7 million individuals suffer a TBI annually [1], and the annual burden of TBI has been estimated at over US $60 billion based upon year 2000 dollars [2]. Yet even these numbers markedly underestimate the incidence and costs. In the US Center for Disease Control and Prevention data [1], sports-related injuries are not listed among the top categories, but some have estimated the incidence of such head injuries at 1.6–3.8 million per year [3]. Globally, the incidence of TBI is also increasing, particularly in developing countries [4].

TBI is a highly complex disorder that includes varying degrees of contusion, diffuse axonal injury, hemorrhage and hypoxia [5,6]. Collectively, these effects induce subsequent

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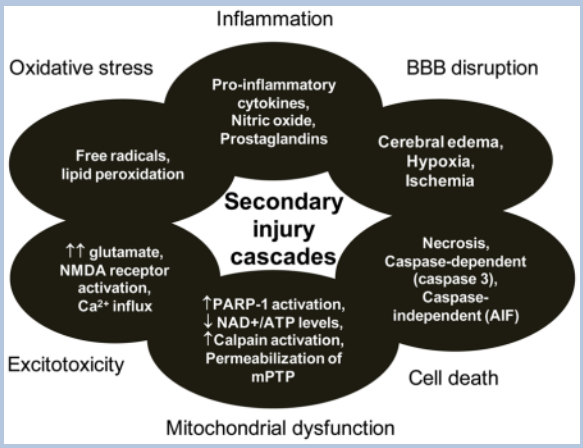
biochemical and metabolic changes that lead to progressive tissue damage and associated cell death [7] (Box 1). Both the early primary events and the delayed secondary alterations contribute to the resulting neurological deficits.

Box 1

Primary and secondary injury

TBI can result in the development of complex neurological deficits and is caused by both primary and secondary injury mechanisms. Primary injury events encompass the mechanical damage that occur at the time of trauma to neurons, axons, glia and blood vessels as a result of shearing, tearing or stretching [5,6]. In addition, secondary injury evolves over minutes to days and even months after the initial traumatic insult and results from delayed biochemical, metabolic and cellular changes that are initiated by the primary event. These secondary injury cascades are thought to account for the development of many of the neurological deficits observed after TBI [7], and their delayed nature suggests that there is a therapeutic window for pharmacological or other treatment (e.g. hypothermia) to prevent progressive tissue damage and improve outcome.



Secondary injury mechanisms include a wide variety of processes such as depolarization, disturbances of ionic homeostasis, and release of neurotransmitters (such as excitatory amino acids), lipid degradation, mitochondrial dysfunction, and initiation of inflammatory and immune processes, among others. Subsequent biochemical events generate large amounts of toxic and pro-inflammatory molecules such as nitric oxide, prostaglandins, reactive oxygen and nitrogen species, and proinflammatory cytokines, which lead to lipid peroxidation, blood-brain barrier (BBB) disruption and the development of edema. The associated increase in intracranial pressure (ICP) can contribute to local hypoxia and ischemia, secondary hemorrhage and herniation and additional neuronal cell death via necrosis or apoptosis. Although each secondary injury mechanism is often considered to be a distinct event, many are highly interactive and may occur in parallel.

Considerable research has sought to elucidate secondary injury mechanisms in order to develop neuroprotective treatments. Although preclinical studies have suggested many promising pharmacological agents, more than 30 phase III prospective clinical trials have failed to show significance for their primary endpoint [8–10]. Most of these trials targeted single factors proposed to mediate secondary injury. But the complexity and diversity of secondary injury mechanisms have led to calls to target multiple delayed injury factors [11–13], either by combining agents that have complementary effects or by using multipotential

drugs that modulate multiple injury mechanisms. The multi-drug approach has long been successfully employed for the treatment of cancer and infectious diseases, but it is less likely to gain traction for neuroprotection because of the costs associated with establishing the efficacy of even a single agent. This recognition has led to the recent emphasis on multipotential treatments, several of which are now in clinical trials and others that are showing considerable promise in preclinical studies (Table 1).

Neuroprotection approaches for both acute and chronic neurodegenerative disorders have historically been dominated by a neuronocentric view, in which modification of neuronal-based injury mechanisms is the primary or even exclusive focus of the neuroprotective strategy. However, increasing evidence in the literature underscores the importance of viewing injury more broadly to include endothelial cells, astoglia, microglia, oligodendroglia and pre-cursor cells. More recent neuroprotection approaches have recognized this complex structure and interplay, emphasizing therapeutic strategies that promote the recovery and optimal functioning of non-neuronal cells in addition to more directly inhibiting mechanisms of neuronal cell death [12,13]. In this review, we describe emerging experimental neuroprotective strategies that show promise for the treatment of TBI and propose criteria for the development of preclinical neuroprotection studies that should improve the probability of successful clinical translation.

Lessons from past failures

The lack of success of neuroprotective drugs clinically has led investigators to identify potential factors contributing to such failures. These include (1) inadequate understanding of secondary injury mechanisms; (2) insufficient preclinical testing in multiple injury models, strains, species (including gyrencephalic), genders and ages; (3) lack of thorough investigation of pharmacokinetics and therapeutic brain concentrations; (4) failure to adequately examine therapeutic window and clinically relevant behavioral outcomes; (5) use of heterogeneous patient populations; (6) inadequate sample size; and (7) inadequate functional outcome measurements and biomarkers. But there are many other critical differences between clinical and preclinical studies that have potential relevance for translation. These include among others: use of anesthesia in animal models with potential drug/anesthetic interactions; use of genetically identical populations and simplistic injury models with high consistency; post-hoc deletion of animal subjects not meeting injury criteria versus the use of an intent-to-treat paradigm clinically. These differences have led some to question the value of animal models or to suggest highly regimented criteria for drugs considered for clinical trials, such as the STAIR criteria established for stroke neuroprotection studies [14,15] (Box 2).

Box 2

Stroke Therapy Academic Industry Roundtable (STAIR) preclinical recommendations

Similar to TBI clinical trials, there have been numerous failed clinical trials with neuroprotective drugs for acute ischemic stroke (AIS). To improve the likelihood of successful translation of experimental neuroprotective agents to clinical trials, the original STAIR I recommendations [15] were developed.

STAIR I recommendations:

1. Therapeutic agents should be tested in both permanent occlusion and transient experimental AIS models
2. Perform adequate dose-response curves for the therapeutic drug

3. Determine the drug's therapeutic window
4. Experimental studies should be blinded, physiologically controlled and reproducible
5. Assess acute and long-term histological and functional outcomes after injury
6. Studies should be performed in rodents and gyrencephalic species

A systematic review of 1026 published neuroprotective therapies for AIS used a checklist derived from STAIR I to provide an overview of the quality of the data available for individual therapies [113]. This study revealed a relationship between increasing study quality score (based on adherence to STAIR I criteria) and therapeutic efficacy [113]. Poor quality studies overestimated efficacy, in part likely reflecting bias from lack of randomization and blinding in experimental studies.

Recently updated STAIR VI recommendations have been published [14]. Modifications include:

1. Studies should eliminate randomization and assessment bias
2. Use a priori defining of inclusion/exclusion criteria
3. Perform appropriate power and sample size calculations
4. Fully disclose potential conflicts of interest
5. Evaluate therapies in male and female animals across the spectrum of ages, and with comorbid conditions such as hypertension and/or diabetes

Unfortunately, in the decade since the publication of the STAIR I recommendations, there has been little progress in successful clinical translation of neuroprotective drugs for AIS. NXY-059, a free radical spin trap agent, was considered by many to have followed the STAIR I recommendations and it showed considerable promise in the SAINT 1 clinical trial resulting in improved outcome in AIS patients [114]. However, a repeat clinical trial (SAINT 2) was a neutral study [115] and cast doubt over its use as a neuroprotective treatment for AIS. Subsequent critical review of these neuroprotection studies attributed its failure to inadequate clinical trial design and a lack of rigorous testing of NXY-059 in preclinical studies [116].

Preclinical challenges

Numerous *in vivo* TBI models have been utilized to address potential mechanisms of secondary injury and to provide proof-of-principle support for specific treatment options. However, methodological concerns have been raised with regard to clinical relevance, including choice of species, strain, or sex. For example, how well do models of brain trauma in rodents reflect injury in higher species? Notably, rodents have relatively small lissencephalic brains with less white matter than humans have. Therefore, it has been suggested that potential therapies should be evaluated in multiple models including higher (gyrencephalic) species [16]. Furthermore, even within a given species, the same model can produce vastly different injury levels and outcomes across various strains [17]. This potentially impacts studies using transgenic animals, in which outcome may reflect the type of backcrossing.

For many preclinical neuroprotection studies, questions arise about the specificity of the treatment target. Proclaimed specific modulators often have additional, and sometimes unexpected, effects on other pathways. Certain experimental strategies can be employed to address this concern, such as the use of structurally different modulators that share similar

modulatory functions or parallel use of knockout models. However, such complementary experimental studies are not commonly performed.

Experimental models of TBI use anesthetized animals. However, anesthetics themselves are drugs that affect injury in different ways, and may serve to enhance or reduce cell death [18]. Moreover, anesthetics may have considerable effects on the actions of the therapeutic drug, and these issues create potential problems for clinical translation.

Animal models often use outcome measures with limited relevance to treatment effects in humans and few animal studies have examined therapeutic windows that correlate to likely treatment times in humans. Indeed, most animal studies generally employ either a pre-treatment or very early post-treatment paradigm (i.e. < 1 hour). In contrast, it is difficult to enter TBI patients into clinical trials before 6 hours, in part because of the informed consent issues. It is also rare for preclinical studies to examine pharmacokinetic or pharmacodynamic profiles of the drugs being examined, or brain levels of drugs in relation to therapeutic actions.

Preclinical study design and statistical analyses also differ considerably from those used clinically. Whereas clinical studies generally employ an intent-to-treat analysis, this is virtually never done pre-clinically. In addition, power analysis is not generally performed to determine the optimal animal sample size; rather, animal numbers are often determined either arbitrarily, or worse, the sample size may be increased incrementally during the study until statistical significance has been achieved. Randomization of treatment and blinding are also important factors that must be included in preclinical experimental design.

Another problem is that clinical trials usually include a range of injury severities, whereas preclinical studies use well-defined, highly controlled animal models of preselected severity. They also preferentially use young healthy animals of a single sex to increase reproducibility, despite the fact that TBI is highly heterogeneous clinically. Furthermore, TBI models typically examine the effects of local contusion or diffuse axonal injury in models that do not include significant secondary insults such as ischemia, hypoxia or associated systemic injuries, despite the fact that these insults are common in clinical TBI [19].

Thus, a number of conceptual and methodological issues have undoubtedly contributed to the difficulty in translating promising neuroprotective treatments from animal to human. To improve the probability of successful clinical translation, we provide recommendations regarding preclinical TBI experimental design and evaluation of neuroprotective agents (Table 2). These build on the STAIR recommendations for preclinical stroke drug development [14,15] (Box 2) and recent NIH-led workshops on effective therapies for TBI [5,11]. However, it is worth noting that adherence to these recommendations may not necessarily result in success. For example, dexanabinol (also known as HU-211) is a multipotential neuroprotective drug [13] that satisfied the majority of the preclinical design criteria outlined in table 2, and was one of the first multipotential drugs entered into clinical trials for head injury. Although dexanabinol was demonstrated to be safe and well tolerated in humans, it failed to improve outcomes in phase III clinical trials and was demonstrated not to be efficacious in the treatment of TBI [20].

Clinical developments to aid bench-to-bedside translation

Disappointing results in clinical trials have led to critical reappraisal of classification of TBI and clinical trial methodology. The current functional classification is based on a 15-point Glasgow Coma Scale (GCS); patients are classified as having mild (14–15), moderate (9–13) or severe (3–8) brain injury. But such delineation is arbitrary and injury severity after

TBI reflects a continuum. Currently, the extended Glasgow Outcome Score (GOS-E), including a larger number of categories, has become the accepted clinical outcome for evaluating TBI treatment effects in clinical trials, but the potential power from inclusion of expanded categories is often lost by dichotomizing into good and bad outcomes. A more comprehensive, evidence-based classification system of patient recovery is clearly needed, and evidence-based secondary outcome measures should be added: cognitive function; quality of life assessments; and physiological, biochemical and imaging surrogate biomarkers. NIH sponsored an expert workshop to reclassify TBI and address pathoanatomical and pathophysiological components [5].

Clinical trial design methodology has also received more critical attention. The International Mission on Prognosis and Clinical Trial Design in TBI (IMPACT) group has addressed ways of improving clinical trial design and analyses to enhance power [21]. Recommendations include maximizing the patient recruitment rates by broadening the inclusion criteria to incorporate the current understanding of the mechanisms of action of the therapeutic intervention being evaluated. Furthermore, the use of new statistical models to incorporate pre-specified co-variate adjustment to mitigate the effects of heterogeneity in TBI patient populations, and the use of ordinal statistical analysis (sliding dichotomy or proportional odds methodology) in clinical trials is recommended and should greatly enhance the statistical efficiency of future studies [21]. Furthermore, recent efforts to revamp data collection standards for TBI have identified Common Data Elements (CDEs), a list of recommended definitions, measurements and tools that would permit data to be better pooled across centers throughout the world (www.nindscommondataelements.org/TBI.aspx). Use of CDEs could potentially result in very large observational data sets that could be used to refine clinical outcome delineation and enhance clinical investigation.

Neuroprotective agents for TBI that have been studied clinically

Given the multifactorial nature of the secondary injury processes after trauma, it is unlikely that targeting any single factor will result in significant improvement in outcome after TBI in human injury. Conversely, simultaneous targeting of several injury factors using multipotential drugs may maximize the likelihood of developing a successful therapeutic intervention to improve outcome in TBI patients. Due to space limitations, only a limited number of promising pharmacological agents currently under study can be detailed in this review. These neuroprotective agents improve outcome in numerous models of experimental TBI, target multiple secondary injury pathways, have clinically-relevant therapeutic windows, and are currently or will imminently be studied in randomized clinical trials for head injury. In addition, we also discuss some emerging multipotential strategies that show promise in preclinical TBI studies and warrant further investigation.

Statins

The 3-hydroxy-3-methyglutaryl coenzyme A (HMGCoA) reductase inhibitors (statins) are inhibitors of cholesterol biosynthesis, but have additional pleiotropic properties [22] that make them potentially attractive neuroprotective agents [23]. At the microvascalture level, statins increase endothelium-derived nitric oxide production [24], reduce vascular inflammation [25], and limit hemorrhagic stroke [26]; after experimental TBI, they reduce post-traumatic hypoperfusion and rebound hyperemia [27]. Statins protect cortical neurons from NMDA-induced excitotoxic death [28], and improve neuronal survival in TBI models [27,29–31]. They decrease apoptosis after trauma [32], and favorably alter the ratio of anti-apoptotic to apoptotic factors [30]. Statins may also promote the growth and differentiation of new neurons [30,33]; increased neurogenesis may reflect upregulation of neurotrophic

factors such as brain-derived neurotrophic factor (BDNF) [33] and vascular endothelial growth factor (VEGF) [34].

Statins exert anti-inflammatory effects, in part by decreasing the formation of isoprenoids. In TBI models, statins have been shown to limit production of inflammatory mediators, glial cell activation and cerebral edema, while increasing blood-brain barrier (BBB) integrity [27,35,36]. They decrease interleukin (IL)-1β [35,36], tumor necrosis factor α (TNFα) [27,35,36], IL-6 [27,35] and intracellular adhesion molecule 1 (ICAM-1) [35] expression levels after TBI. Inhibition of the toll-like receptor 4 and the nuclear factor κB (NFκB) signaling pathways are potential mechanisms by which statins may modulate the inflammatory response [35].

Preclinical studies demonstrate that statins target multiple secondary injury pathways and improve functional outcome after TBI [27,29–32,35,36]. Furthermore, the therapeutic window for this class of drugs is relatively large, with treatment 24 hours after TBI resulting in long-term functional improvements and reduced neuronal cell loss [29,30]. Importantly, statins are well tolerated, easy to administer, have well-defined side effects, are easily monitored, and have a long clinical track record in critically ill patients [37]. A small prospective, randomized, double-blind clinical trial in TBI has been performed using rosuvastatin. Treatment showed a modest improvement in TBI-associated amnesia and disorientation time outcomes [38]; other phase II clinical trials to evaluate rosuvastatin and atorvastatin in the treatment of head injury are planned.

Progesterone

Progesterone is a neurosteroid whose receptors are expressed in the central nervous system (CNS) of both males and females [39]. Neuroprotective effects for progesterone have been reported in experimental spinal cord injury (SCI) [40], stroke [41] and TBI [42]. Roof *et al*. [43] observed that female rats performed better than males in the Morris water maze after experimental TBI and that progesterone-treated male rats were less impaired in the task than vehicle-treated animals [44]. These effects were associated with a reduction in neuronal cell death [45]. Progesterone attenuates glutamate excitotoxicity [46], modulates apoptotic pathways [47,48], and decreases diffuse axonal injury [49]; it also reduces membrane lipid peroxidation [50], possibly by upregulating superoxide dismutase [51]. Treatment limits inflammation after TBI, attenuating NFκB, p65 and TNFα expression [52,53]. Progestesterone also reduces edema after injury [49,54] through mechanisms that may include inhibition of Na^+,K^+-ATPase, modulation of vasopressin [13,55], or maintaining BBB function by upregulating P-glycoprotein [56].

Although progesterone has been shown to have neuroprotective activity in models of experimental stroke, SCI and TBI, recent reports question its effectiveness in trauma models. Importantly, a systematic review of progesterone treatment in CNS injury raised concerns about the methodological quality of the TBI studies, and quantitative evaluation revealed possible experimental bias in these studies [57]. In addition, a highly regarded neurotrauma group failed to observe any protective effects of progesterone, at doses reported to be effective by others, in well characterized models of either TBI or SCI [58,59]. Moreover, a clinically relevant therapeutic window has not been examined in most published neurotrauma studies, with the majority evaluating very early treatment times (i.e. 0–2 hours) after injury [57].

Despite the limitations of the preclinical data, two randomized, double-blind, placebo-controlled phase II clinical trials for progesterone have been conducted [60,61]. Although these trials used different doses and treatment regimens, both indicated trends toward improved outcome in progesterone treated patients. However, the number of patients studied

was limited, and randomization in the ProTECT trial[60] was 3:1, resulting in relatively few vehicle treated controls. Based upon the positive trends in the phase II studies, two phase III multi-center clinical trials are now under development. The ProTECT III trial (NINDS/NIH) will examine intravenous (iv) progesterone initiated within 4 hours of injury and continued for 72 hours in 1140 patients with moderate to severe TBI [62]. The SyNAPSE phase III trial (BHR Pharma) is a global, multi-center trial of BHR-100 (iv progesterone infusion) in approximately 1200 severe TBI patients, with treatment to be initiated within 8 hours of injury [62].

Cyclosporine A

There are significant impairments of aerobic metabolism early after TBI [63]. Mitochondrial failure leads to energy and ionic imbalances, reduced brain ATP levels, changes in mitochondrial permeability transition, release of cytochrome c and induction of pro-apoptotic events [64]. The immunosuppressant drug cyclosporine A (CsA) attenuates mitochondrial failure by binding to cyclophilin D and stabilizing the mitochondrial permeability transition pore (mPTP) [65]. Treatment with CsA reduced axonal damage in diffuse axonal injury models [66,67] and decreased lesion size following controlled cortical impact (CCI) TBI [68,69]. Improved outcome was associated with preserved mitochondrial function [70] or structural integrity [67,71]. CsA attenuates lipid peroxidation and free radical oxidative damage to mitochondrial proteins [71,72]. Given these multipotential effects and long therapeutic window (up to 24 hours [68]), CsA appears to be an attractive candidate for clinical investigation.

A prospective randomized, placebo-controlled, double-blinded clinical trial of CsA was performed at two centers for severe human TBI. Patients treated with CsA showed significantly lower lactate/pyruvate ratios [73], which may reflect improved metabolism [63]. Larger phase III clinical trials for CsA are in preparation. Potential advantages for CsA are that it is FDA-approved for other uses and off-patent. However, CsA shows relatively poor brain penetration, has a biphasic drug-response curve, and prolonged use adversely impacts the immune system [11].

Emerging neuroprotective therapies

Diketopiperazines

Diketopiperazines are cyclized dipeptides that were developed through a rational drug design program based on the tripeptide thyrotropin-releasing hormone (TRH) [74]. TRH and TRH analogs inhibit multiple secondary injury factors and processes [74]. They were shown to be highly neuroprotective in experimental neurotrauma across many laboratories and a small clinical randomized study of TRH in human SCI was promising [75]. Four structurally different diketopiperazines demonstrated significant neuroprotective properties both *in vitro* and in animal TBI studies [76]. One of these (35b) showed effectiveness across TBI models and species. In neuronal cell cultures, 35b provided neuroprotection in multiple models of necrotic and apoptotic cell death [77]. Intravenous administration of 35b reduced both lesion volume and improved functional recovery after fluid percussion injury (FPI) in rats and CCI in mice [77,78]. The therapeutic window was at least 8 hours. Treatment also significantly reduced apoptotic cell death [77]. Effects appeared to be pleiotropic, with treatment reducing multiple potential secondary injury factors (cyclins, calpains, cathepsin), while up-regulating various endogenous neuroprotective and neurotrophic factors (BDNF, HSP-70, HIF-1) [76,79]. In addition, data are available regarding pharmacokinetics, brain penetration after systemic administration and preclinical toxicology in the rat. Similar pleiotropic neuroprotective effects were reported with another diketopiperazine - cyclo-L-glycyl-L-2-allylproline (NNZ 2591) - in rats with hypoxic-ischemic brain injury [80]. NNZ 2591

treatment improved functional recovery and long-term histological outcomes, and reduced caspase-3 mediated apoptosis and microglial activation [80]. Given their multipotential neuroprotective effects in experimental TBI models, their clinically relevant therapeutic window, and their safety profile, the diketopiperazines are attractive candidates for further clinical investigation.

Substance P (SP) Antagonists

SP is released early following trauma as part of a neurogenic inflammatory response [81]. Inhibition of post-traumatic SP activity, either by preventing SP release or by antagonism of the neurokinin-1 receptor, reduced inflammation associated with acute TBI and maintained the integrity of the BBB [81]. Furthermore, administration of SP antagonists decreased BBB permeability and edema formation, reduced axonal injury, enhanced neuronal survival and improved behavioral outcomes following experimental TBI [82,83]. These promising preclinical data warrant further investigation in additional animal models and species, along with evaluation of the therapeutic window.

SUR1-regulated $NC_{Ca\text{-}ATP}$ Channel Inhibitors

Edema and progressive secondary hemorrhage are important secondary injury mechanisms that contribute to neurological impairments in patients after TBI. Recent studies suggest that upregulation of sulfonylurea receptor 1 (SUR1)-regulated $NC_{Ca\text{-}ATP}$ channels in microvascular endothelium play a key role in these secondary injury pathways [84]. Pharmacological blockade using the SUR1 inhibitor glibenclamide reduced edema, secondary hemorrhage, inflammation, apoptosis, and lesion size in experimental TBI and subarachnoid hemorrhage models [85,86]. Furthermore, glibenclamide treatment improved functional recovery after TBI [86]. These data indicate the therapeutic potential of targeting SUR1-regulated $NC_{Ca\text{-}ATP}$ channels in TBI, and a prospective clinical trial of glibenclamide in TBI is anticipated to start soon.

Cell Cycle Inhibitors

Upregulation of cell cycle proteins occurs in both mitotic (astrocytes and microglia) and post-mitotic (neurons, oligodendroglia) cells after CNS injury, and is associated with caspase-mediated neuronal apoptosis and glial proliferation after TBI [87]. Cell cycle inhibitors have been extensively evaluated in cancer. Inhibitors such as flavopiridol, a semi-synthetic flavonoid, and the purine analogues, roscovitine and olomoucine, exert powerful neuroprotective effects in various models of neuronal cell death [88–90], as well as inhibitory effects on the proliferation and activation of astocytes and microglia [87,88,91]. Cell cycle inhibition *in vivo* is strongly neuroprotective. ICV administration of flavopiridol (an inhibitor of all major cyclic-dependent kinases) after FPI in rats reduced lesion volume by approximately 70% and improved cognitive and sensorimotor recovery to the level of uninjured controls [87]. Caspase-mediated neuronal cell death after TBI was nearly completely attenuated. In addition, flavopiridol markedly reduced glial cell activation, and these changes were associated with suppression of cell cycle proteins in neurons, astrocytes, and microglia. Furthermore, delayed administration of flavopiridol had similar neuroprotective effects; systemic treatment (intraperitoneal (ip)) given 24 hours post-trauma significantly reduced lesion volumes [88]. Roscovitine, a more selective cell cycle inhibitor, had similar neuroprotective actions. In addition to improving functional recovery and reducing the lesion, central administration (ICV) of roscovitine reduced astrogliosis and produced a marked inhibition of microglial-mediated neuroinflammation [91]. Protective effects of cell cycle inhibitors have also been demonstrated after experimental SCI [92] and stroke [93]. Importantly, several of these cell cycle inhibitors have been well studied in randomized clinical trials for cancer [94]. Although they are toxic when administered chronically, cell cycle inhibitors have required only single dose administration to exert

therapeutic effects in TBI. Given their clinically relevant therapeutic window and extensive clinical evaluation, cell cycle inhibitors such as flavopiridol or roscovitine appear to be attractive candidates for future clinical investigation.

Other promising targets for pharmacological intervention

Preclinical studies that use pharmacological means to target apoptosis after TBI have shown promise. Caspase inhibitors, such as the tetrapeptide caspase-3 inhibitor (z-DEVD-fmk), improve neurological outcome, reduce the lesion size and inhibit caspase enzyme activity after experimental TBI [95,96]. Similarly a pan-caspase inhibitor improved functional recovery after TBI [97], and central administration of the pan-caspase peptide inhibitor Boc-aspartyl fluoromethylketone attenuated mitochondrial release of cytochrome c and delayed the loss of brain tissue after TBI in rats [98]. A potential problem with many caspase inhibitors is their limited ability to penetrate the BBB. However, Boc-aspartyl fluoromethylketone crosses the BBB, reducing ischemic brain damage after systemic administration [99], and may have similar effects in models of TBI.

Inhibitors of poly (ADP-ribose) polymerase (PARP) are also neuroprotective in experimental models of TBI [100–102]. They limit caspase-independent apoptosis mediated by apoptosis inducing factor (AIF) [103] and also markedly inhibit microglial activation and the release of inflammatory factors after CNS injury [104–106].

Another area of intense preclinical research is the development of pharmacological agents that modulate the inflammatory response after TBI [107]. Anti-inflammatory treatments, such as minocycline, are neuroprotective in experimental models of TBI [108], and administration of anti-inflammatory cytokines (e.g. IL-10) or interleukin-1 receptor antagonist (IL-1ra) confer significant neuroprotection and result in improved functional recovery after experimental TBI [109–112].

Conclusion

TBI is a highly complex disorder, which is characterized by multiple interacting secondary injury cascades. The focus on highly selective “laser-guided” neuoprotective strategies has recently given way to the concept of multipotential drugs that modulate multiple secondary injury pathways. The potential limitations of using single models and species for preclinical screening of neuroprotective agents has been increasingly underscored, as have the methodological differences between clinical and preclinical trials. At the same time, there has been increasing attention directed toward methodological issues in clinical trial design and analysis. In the future, it will be important to better facilitate bidirectional translational research between preclinical and clinical investigators, which should serve to improve both approaches to animal modeling and the design of clinical trials. Future advances in clinical data sharing (using common data elements) should improve TBI classification in ways that may lead to delineation of specific patient subgroups that may benefit from better targeted neuroprotective strategies.

Glossary

Primary injury	the immediate and irreversible tissue damage localized in areas that absorb mechanical energy following the initial traumatic insult to the brain. Primary brain damage includes shearing of white-matter tracts/ diffuse axonal injury, focal contusions and hematomas

Secondary injury	delayed and reversible molecular and cellular pathophysiological mechanisms that evolve and expand around the primary injury site over hours and days after TBI resulting in further grey and white matter damage. Secondary brain injury is responsible for the development of neurological impairments following TBI
Multipotential drugs	therapeutic agents that modulate multiple secondary injury mechanisms to improve histological and functional outcomes after TBI
Gyrencephalic	brains of humans and higher species, in which the cerebral cortex has convolutions (gyri and sulci), in contrast to the lissencephalic (smooth) brains of small mammals such as the rodents
Intent-to-treat	human randomized clinical trials generally employ an intent-to-treat paradigm, where all cases that should have received the treatment are included in the statistical analysis even when they fail to receive the drug or the right dose. All cases allocated to each arm of the trial are analyzed together as representing that treatment arm, regardless of whether they received or completed the prescribed drug treatment regimen
Backcrossing	breeding a transgenic animal with one of its parents or an individual genetically similar to its parent, in order to achieve offspring with a genetic identity which is closer to that of the parent

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Table 1

Multipotential drugs for TBI

Multipotential drug	Targeted secondary injury mechanism	Reference
Statins	Excitotoxicity	[28]
	Apoptosis	[30,32]
	Inflammation	[27,35,36]
	Edema	[27,35]
	BBB disruption	[35]
Progesterone	Excitotoxicity	[46]
	Apoptosis	[47–49]
	Oxidative stress	[50]
	Inflammation	[52,53]
	Edema	[49,54]
Cyclosporine A	Mitochondrial dysfunction	[64,66,70,71]
	Calpain activation	[67,71]
	Apoptosis	[64]
	Oxidative stress	[71,72]
Diketopiperazine	Apoptosis	[76,77,80]
	Calpain and cathepsin activation	[76,79]
	Inflammation	[80]
Cell cycle inhibitors	Apoptosis	[88–91]
	Inflammation	[87,88,91]
PARP inhibitors	Apoptosis	[103]
	Inflammation	[104–106]

Table 2

Design Criteria for PreclinicalDevelopment of Pharmacological Agents for TBI

Design Criteria for PreclinicalDevelopment of Pharmacological Agents for TBI
• Perform drug dose–response curves and evaluate efficacy throughout the spectrum of injury severities.
• Use power analysis to determine sample size, randomize TBI surgeries and drug treatments, and use blinding in all histological and functional outcome testing.
• Demonstrate specificity of the pharmacological agent by using structurally different modulators and parallel use of knockout technology and pharmacological antagonists.
• Perform therapeutic window studies for the pharmacological agent to include a clinically relevant delayed treatment time point (>8 hours post TBI).
• Use both histological and functional outcome measurements (>14 days post TBI) to demonstrate sustained long-term effects of the treatment.
• Examine the pharmacokinetic profile of the drug and brain concentrations associated with treatment efficacy.
• Include clinically relevant physiological monitoring during and after TBI surgery and treatment.
• Evaluate the pharmacological agent in both genders and across the spectrum of ages.
• Evaluated the pharmacological agent in multiple TBI models and species, preferably including a higher (gyrencephalic) species.
• Replicate the therapeutic effects of the drug across laboratories