Neuroprotection targets after traumatic brain injury

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Purpose of review

The scarcity of pharmacological neuroprotective treatments for traumatic brain injury is a concern being targeted on various fronts. This review examines the latest treatments under investigation.

Recent findings

In the last 12–18 months, no drug has completed phase III clinical trials as a clearly proven method to treat traumatic brain injury. While the drugs work in rodents, when they make it to clinical trial they have failed primarily due to negative side-effects. Those still in trial show promise, and even those rejected have undergone modifications and now show potential, e.g. second-generation *N*-methyl-D-aspartic acid and α-amino-3-hydroxy-methyl-4-isoxazolyl-propionic acid receptor antagonists, calpain inhibitors, and cyclosporine A analogues. Also, several drugs not previously given much attention, such as the antibiotic minocycline, estrogen and progesterone, and a drug already approved for other diseases, erythropoietin, are being examined. Finally, a treatment generating some controversy, but showing potential, is the application of hypothermia to the patients.

Summary

Clearly, finding treatments for traumatic brain injury is not going to be easy and is evidently going to require numerous trials. The good news is that we are closer to finding one or more methods for treating traumatic brain injury patients.

Keywords

antagonist, hypothermia, inhibitor, neuroprotection, traumatic brain injury

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Abbreviations

AMPA	α-amino-3-hydroxy-methyl-4-isoxazolyl-propionic acid
CsA	cyclosporine A
EPO	erythropoietin
GCS	Glasgow Coma Score
MTBI	mild traumatic brain injury
NMDA	*N*-methyl-D-aspartic acid
TBI	traumatic brain injury

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Introduction

Traumatic brain injury (TBI), a significant health problem, represents a potentially catastrophic debilitating medical emergency with poor prognosis and long-term disability. Each year in the US at least 1.4 million people seek medical help for a TBI, of which about 50 000 die, 235 000 are hospitalized, and 1.1 million are treated and released from an emergency department [1]. An estimated 90 000 of these patients will suffer permanent impairment from their injury and more than half will experience at least short-term disability. Yet with all these potential patients, there is no clinically proven therapy.

Mild traumatic brain injury – a silent epidemic

TBI severity is classified based on Glasgow Coma Score (GCS). Of the 1.4 million TBIs reported annually [1], about 10–25% are severe (GCS 3–8), while the rest are moderate (GCS 9–12) or mild (GCS 13–15) (MTBI) [2]. However, MTBI is under-diagnosed and occurrences are underestimated because many sufferers do not seek medical attention. MTBI concussion, one of the most common neurological disorders [3], occurs when an impact or forceful motion of the head results in a brief alteration of mental status, such as confusion or disorientation, or brief loss of memory or consciousness. Even such brief alterations in mental status can, however, inflict profound and persistent impairment of physical, cognitive and psychosocial functioning [4]. MTBI is often referred to as a 'silent epidemic' because its neurological sequelae are nonspecific and it is a common occurrence in the general population [5,6]. Many sufferers and healthcare providers fail to recognize the potential severity of a brief loss of consciousness [7]. Often, individuals with MTBI do not receive medical care at the time of injury, but see their primary care physician days, weeks or even months after the injury with complaints of persistent symptoms [7,8]. Of the total

annual estimated costs of US$56 billion associated with TBI, US$16.7 billion are for MTBI [9]. These estimates do not include costs for lost productivity or quality of life.

Blast-induced brain injury

The leading cause of combat casualties is brain injury, with an estimated 15–25% of all injuries sustained in 20th century conflicts [10]. An emerging trend in modern warfare is the dramatic increase of blast-induced brain injuries due to supersonic over-pressurization shock waves generated by high-order explosives. The blast injuries are generated as the wave propagates through the body damaging the gas–fluid interfaces [11]. The most serious damage is inflicted on internal gas-filled structures such as the lungs, gastrointestinal tract and middle ear. Air emboli can also form in blood vessels, causing cerebral infarcts when they travel to the brain. The brain, a soft tissue, is believed to be vulnerable to the direct impact of the shock wave as well. As insurgents in Iraq and Afghanistan continue to use improvised explosive devices against American troops, closed head injuries significantly outnumber penetrating ones amongst patients being treated at the Walter Reed Army Medical Center. All blast exposed casualties are now routinely evaluated for brain injuries – 59% are diagnosed with TBI, of which 56% are considered moderate or severe [12].

Different neuroprotective mechanisms – different targets

There are numerous targets with their attending neuroprotective mechanisms for the treatment of TBI. Although there are many targets, each with their own drug treatments under investigation [13••], in this review we will focus on those receiving the most attention and have proceeded the furthest in terms of clinical relevance (Table 1).

Table 1 Classes of neuroprotectants

Class	Mechanism	Treatments still under investigation
NMDA receptor antagonists	antagonists of major ionotropic NMDA-subtype glutamate receptor, suppressing excitotoxic responses	aptiganel, eliprodil, memantine, nitromemantines, traxoprodil, ACEA-1416, arcaine
AMPA receptor antagonists	antagonists of ionotropic AMPA-subtype glutamate receptor, suppressing excitotoxic response	zonampanel (YM872), BIIR-561-CL (irampanel)
Necrosis inhibitors	calpain inhibitors (also inhibit some forms of apoptosis)	MDL28170, SJA6017, SNJ-1945
Apoptosis inhibitors	pan-caspase inhibitors	M826, MX1013, IDN-6566, IDN-5370, minocycline, dexamethasone
Necroptosis inhibitor	prototype inhibitor of caspase-independent cell death	necrostatin-1
Immunophilin ligands	ligands that bind to immunophilin proteins that might suppress calcineurin activity or mitochondria permeability transition pore	cyclosporine A, DEBIO-025, UNIL025, NIM811, FK506
Ovarian hormones	neuroprotective likely through brain-specific hormone receptor subtype(s)	estrogen, progesterone
Erythropoietin	hypoxia-induced cytokine/hormone that suppress neuronal apoptosis by acting on brain erythropoietin receptors	recombinant human erythropoietin
Hypothermia	body or brain cooling to reduce metabolic load on injured brain	33°C for at least 48 h

There are a number of mechanisms that can be targeted by neuroprotectants. This lists the most prominent targets where investigation is currently quite active, and the drugs and treatments that still have potential. AMPA, α-amino-3-hydroxy-methyl-4-isoxazolyl-propionic acid; NMDA, *N*-methyl-D-aspartic acid.

N-Methyl-D-aspartic acid receptor antagonists

N-methyl-D-aspartic acid (NMDA) receptor-linked glutamate excitotoxicity has been shown to contribute to neural injury in TBI. Although the early noncompetitive NMDA antagonists, phencyclidine and MK801, were shown to be neuroprotective against TBI in rats [14,15], they unfortunately were not clinically acceptable. New drugs, however, have been or are currently being tested.

A glutamate antagonist (competitive NMDA receptor blocker) selfotel (CGS 19755) was abandoned during phase III trials for stroke and TBI after interim analysis showed no benefit [16••]. Similarly, phase III trials of ion channel-blocking NMDA receptor noncompetitive antagonists, aptiganel and eliprodil, were terminated early when safety concerns became an issue and the results were no better than neutral, so the data remain inadequately reported [16••].

Other drugs under study include memantine, a phase III clinically tolerated effective agent in treating Alzheimer's disease, which is currently in trials for additional neurological disorders. Combinatorial drugs called nitromemantines were developed to use memantine as a homing signal to target nitric oxide in hyperactivated NMDA receptors in the hope they would be able to avoid some of the systemic side-effects. These second-generation memantine-derivative therapeutics were designed to be activated under pathologically conditions and, in preliminary studies, appear to offer better neuroprotection [17••]. Traxoprodil (CP-101606) antagonist is highly selective for the NR2B subunit of the NMDA receptor [18]; ACEA-1416, an analog of ACEA-1021 [19], and arcaine, an analog of agmatine [20], have been shown to be neuroprotective in animal models of brain injury and ischemia, and appear to better tolerated.

α-Amino-3-hydroxy-methyl-4-isoxazolyl-propionic acid receptor antagonists

The activation of α-amino-3-hydroxy-methyl-4-isoxazolyl-propionic acid (AMPA) receptors provides the initial membrane depolarization to relieve the magnesium block – a prerequisite for the activation of NMDA receptors. Inhibitors for AMPA receptors have not had the same reported side-affects as the NMDA receptor antagonists, making them a more agreeable target. Second-generation noncompetitive AMPA receptor antagonists such as GYKI 53405 and talampanel have been shown to be neuroprotective in experimental TBI or stroke models [21,22], but failed to advance successfully in clinical trails. A new noncompetitive AMPA antagonist zonampanel monohydrate (YM872), which is also neuroprotective in rats [23], is now in phase II clinical trial for treating stroke patients. Like the Gyki compound, the oxadiazole BIIR 561 CL (irampanel) is also a noncompetitive antagonist with neuroprotective effects in rats, but it binds to a different site on the receptor. It also has an additional effect – it has been shown to block neuronal voltage-gated sodium channels [24].

Calpain inhibitors

Over-activation of cellular proteases is another key response in brain cells after physical or chemical stresses. Traumatic or ischemic insult which induces massive release of glutamate from damaged synapses can lead to activation of glutamate receptor-associated and voltage-dependent calcium channels. Such influx of calcium ions directly activates the calcium-activated cytosolic protease calpain. In fact, calpains are prominently activated in pro-necrotic cell injury, but are also activated during neuronal apoptosis [25]. Calpains, as proteases, have the capability to degrade key structural brain cell proteins leading to tissue auto-digestion.

Calpain inhibitors have been demonstrated to be neuroprotective in many ischemic and TBI animal models [26]. Treatment aimed at downstream neuropathological events could provide a longer window of opportunity for effective intervention and therefore be valuable for more patients. In the rat TBI model, calpain proteolysis is initiated within the first few minutes after injury, but peak activity can persist for hours [27,28] or even several days in mild injury [29]. Indeed, studies using calpain inhibitors, MDL-28170 and SJA6017, in models of cerebral ischemia [30] and TBI [31] indicate a potential therapeutic window of at least 3–6 h. Calpain inhibitors may have a further advantage over glutamate receptor antagonists and calcium channel blockers in that calpain exists predominantly as an inactive proenzyme under normal physiological conditions, and only becomes significantly activated under pathological conditions. Therefore, it would be reasonable to assume that calpain inhibition would not lead to any untoward adverse events. On the other hand, glutamate receptors play a critical neurotransmitter role in and outside of the central nervous system, and therefore their inhibition could be expected to have profound side-effects. Indeed, they have also been shown to have significant psychotomimetic outcomes [32].

Drawbacks of calpain inhibitors include relative low solubility of this class of compounds, and lack of metabolic stability and optimal pharmacokinetic profile. Recently, the chemically optimized calpain inhibitor, SNJ-1945, was reported to have significantly improved solubility and metabolic pharmacokinetic profile [33]. We now await further advancement of this class of agents.

Caspase inhibitors

Parallel with the calpain activation and necrosis, brain cells may undergo physical or chemical homeostatic perturbations that may lead to apoptosis. One major biochemical hallmark of apoptosis is the activation of the caspase family of proteases. The major executioner in this family is caspase-3, which has the capability to degraded key structural proteins leading to delayed neuronal cell death. Caspase inhibitors have been demonstrated to be neuroprotective in many animal models of ischemic and TBI [26]. For example, the potent pan-caspase inhibitor M826 is neuroprotective against neonatal hypoxic–ischemic brain injury [34], while MX1013 reduced cortical damage by approximately 50% in a model of brain ischemia/reperfusion injury [35]. Recently, the chemically optimized and drug-like caspase inhibitor, IDN-6556, was reported to suppress apoptosis in a model of hepatic injury [36•]. This drug is currently in clinical phase II trial for liver transplants as an antihepatic apoptosis agent. Although IDN-6556 has not been tested in experimental TBI models, it would be interesting to see if its antiapoptotic effects extend to TBI and whether it can cross the blood–brain barrier. Another caspase inhibitor produced by the same company, IDN-5370, was found to be protective against apoptosis induction in cortical and synaptic neurons, and reduced infarct size in rodent cardiac ischemia/reperfusion models by more than 50% [37]. Minocycline, a broad-spectrum tetracycline antibiotic member, was found to inhibit cytochrome *c* release. In use for more than 30 years, it was specifically designed to cross the blood–brain barrier. It has recently been reported this antibiotic can protect brain cells in animal models of diseases such as acute brain injury, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, stroke, etc. The drug is currently in early clinical trials [37]. Finally, dexamethasone has been found to decrease caspase-3 activation in meningitic animals, demonstrating that dexamethasone can decrease acute brain injury in a rat model of bacterial meningitis as measured by neurobehavioral performance [38•].

Necroptosis inhibitor

A new nonapoptotic death pathway, termed necroptosis, characterized by necrotic cell death morphology and activation of autophagy, was recently described as a contributor to ischemic injury. In the study a specific and potent small-molecule inhibitor of necroptosis, necrostatin-1, was identified by its ability to block a critical step in necrotic cell death induced by death receptor activation even in the presence of caspase inhibitors, thereby offering a new neuroprotective and therapeutic target for stroke [39•].

Immunophilin ligands

Mitochondrial dysfunction, leading to increased mitochondrial permeability transition pore openings, is a hallmark of neuronal cell perturbation in both pro-necrotic and pro-apoptotic challenges. Cyclosporine A (CsA) and analogues have been shown to bind to the mitochondrial-specific cyclophilin D, a component of the permeability transition pore stabilizing the mitochondrial permeability transition, as well as to calcineurin. CsA was found neuroprotective in an experimental model of diffuse brain injury [40]. One possible drawback to CsA is that it is immunosuppressive and could be counter-indicated in TBI patients. Recent data, however, showed that nonimmunosuppressive CsA analogues such as DEBIO-025 [41], UNIL025 and NIM811 [42,43], with the latter two determined to be more potent than CsA, are also neuroprotective and thus may be a good candidates for TBI therapy.

Another immunophilin ligand, FK506, which does not stabilize mitochondrial permeability transition, attenuates TBI impaired axonal transport, although it fails to attenuate neurofilament compaction [44]. FK506 apparently operates by complexing with FK-binding proteins and calcineurin, interacting at a completely different site on calcineurin from CsA and thereby providing some measure of neuroprotection [45].

Ovarian hormones: estrogen and progesterone

It has been well established that estrogen and progesterone provide gender-based neuroprotective effects in ischemic and TBI [46•]. Estrogen receptor subtype α, found in the brain [47•], is now believed critical in mediating neuroprotection. Progesterone appears particularly effective in protecting against lipid peroxidation following TBI in rats [48]. The number of studies continues to grow on the beneficial influences on neuronal injury of these steroids and their actions appear to be exerted on multiple processes. The mechanisms by which these steroids mediate these effects are, however, still uncertain [49••]. It is, nonetheless, possible that the combined use of estrogen and progesterone (or their more refined analogues) could be a viable therapy against TBI.

Erythropoietin

Erythropoietin (EPO) has been a surprising entry into the stable of possible neuroprotective drugs. Since nearly all brain cells, including neurons, astrocytes, oligodendrocytes, microglia and the endothelial cells lining the capillaries [50], appear capable of expressing EPO and its receptor [51•] when induced by hypoxia, it appears to offer multifaceted protection from deleterious stimuli such as hypoxia, excess glutamate, AMPA, serum deprivation or kainic acid [50] exposure. In rodent models of ischemic stroke with an increase in apoptotic lesions [52•], a regime of EPO reduces infarct volume, and prevents behavioral abnormalities, cognitive dysfunction and brain atrophy [53•]. In general, EPO improves functional outcome in animal models with subarachnoid and intracerebral hemorrhage, TBI [50], and spinal cord injury. EPO, with its convenient 6-h therapeutic window and its improved safety profile with the advent of the recombinant human form, has been employed in a limited therapeutic trial for stroke. The results were promising enough so that a larger multicenter phase II/III trial has been initiated in Germany [54••].

Hypothermia

Hypothermia, while not a drug, is a medical treatment that has recently shown some positive results (e.g. [55]) in single-center trials, but one multicenter clinical trial failed to clearly to show a positive effect leading to some controversy [56•,57•]. This multicenter study has been criticized on several points, including trial methodology, design and intervention application, group comparison, and intercenter variations. What was evidentially unambiguous is that hyperthermia occurs in the majority of the brain-injured patients, and that a relationship between hyperthermia and poor outcome exists. It is also clear that hypothermia treatment reduces intracranial hypertension [58••]. A new multicenter phase III study has recently completed enrollment and the data are currently being analyzed.

Conclusion

TBI represents a major central nervous system disorder without any clinically proven therapy. In this review, various pharmaceutical agents or treatments have been shown to have beneficial effects in animal models of TBI and even some cases on human patients. The past 10 plus years have, however, witnessed numerous failures in clinical drug trials for the treatment of TBI in humans. This indicates just how difficult it is to translate promising preclinical data into clinical successes [59]. In retrospect, a number of key missing components can be identified in these clinical trials and need to be included in future trials: (1) stronger preclinical animal efficacy data (with positive results from at least two animal models of TBI), (2) advancing drug candidates with an extended therapeutic window of at least 3–4 h (i.e. drug still shows neuroprotection

even when given 3–4 h post-TBI), (3) better overall clinical trial design and (4) incorporation of clinical TBI biomarkers as guidance for drug response [60•]. These are not small challenges, but the diversity and novelty of emerging neuroprotective agents give TBI researchers and clinicians a sense of much-needed optimism.

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