Neuroprotection targets after traumatic brain injury

Kevin K.W. Wang\textsuperscript{a,b,c}, Stephen F. Larner\textsuperscript{a,b}, Gillian Robinson\textsuperscript{c} and Ronald L. Hayes\textsuperscript{a,b,c}

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

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).

**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°].

### 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, zonampanel (YM8722), BIIR-561-CL (irampanel) |
| AMPA receptor antagonists| antagonists of ionotropic AMPA-subtype glutamate receptor, suppressing excitotoxic response |                                          |
| 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 necrostatin-1 |
| Necroptosis inhibitor    | prototype inhibitor of caspase-independent cell death | cyclosporine A, DEBIO-026, UNL025, NIM811, FK506 |
| Immunophilin ligands     | ligands that bind to immunophilin proteins that might suppress calcineurin activity or mitochondria permeability transition pore |                                        |
| 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, alpha-amino-3-hydroxy-5-methyl-4-isoxazolyl-propionic acid; NMDA, N-Methyl-D-aspartic acid.

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-effects 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 trials. 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 neuro-pathological 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 3–6h. 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 [45].

**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 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 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.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 620–623).

1 Langlois JA, Marr A, Mitchko J, et al. Tracking the silent epidemic and educating the public: CDC’s traumatic brain injury-associated activities under the TBI Act of 1996 and the Children’s Health Act of 2000. J Head Trauma Rehabil 2005; 20:196–204.

2 Jager TE, Weiss HB, Cohen JH, Pepe PE. Traumatic brain injuries evaluated in US emergency departments, 1992–1994: Acad Emerg Med 2006; 7:134–140.

3 Kurtzke JF, Kurland LT. The epidemiology of neurologic disease. In: Joynt RJ, editor. The epidemiology of neurologic disease. Philadelphia: Lipincott; 1992; pp. 80–88.

4 Binder LM. A review of mild head trauma. Part II: clinical implications. J Clin Exp Neuropsychol 1997; 19:432–457.

5 Iversen GL, McCracken LM. ‘Postconcussive’ symptoms in persons with chronic pain. Brain Inj 1997; 11:783–790.

6 Wessely S, Nimnuan C, Megginson W, et al. The TBI Act of 1996 and the Children’s Health Act of 2000. J Head Trauma Rehabil 2003; 20:269–278.

7 Buki A, Koizumi H, Povlishock JT. Moderate posttraumatic hypothermia decreases early calpain-mediated proteolysis and concomitant cytoskeletal compromise in traumatic axonal injury. Exp Neurol 1999; 159:319–328.

8 Ringger NC, Tolentino PJ, McKinsey DM, et al. Effects of injury severity on regional and temporal mRNAs expression levels of calpains and caspases after traumatic brain injury in rats. J Neurotrauma 2004; 21:829–841.

9 Pretreatment with phencyclidine, an N-methyl-D-aspartic acid antagonist, attenuates long-term behavioral deficits after delayed administration in a mouse model of diffuse head injury. J Neurotrauma 2001; 18:1229–1240.

10 Olney JW, Labuyere J, Wang G, et al. NMDA receptor neurotoxicity: mechanism and prevention. Science 1991; 254:1515–1518.

11 Shirasaki Y, Miyashita H, Yamaguchi M, et al. Exploration of orally available calpain inhibitors: pepidyl alpha-ketoamides containing an amiphile at P9 site. Bioorg Med Chem 2001; 13:4473–4484.

12 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.

13 Yang W, Guastella J, Huang JC, et al. Exploration of orally available calpain inhibitors: pepidyl alpha-ketoamides containing an amiphile at P9 site. Bioorg Med Chem 2001; 13:4473–4484.

14 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.

15 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.

16 Furukawa T, Hoshino S, Kobayashi S, et al. The glutamate AMPA receptor antagonist, YM872, attenuates cortical tissue loss, regional cerebral edema, and neurological motor deficits after experimental brain injury in rats. J Neurotrauma 2005; 22:269–278.

17Swift TL, Wilson SL. Misconceptions about brain injury among the general public and nonexpert health professionals: an exploratory study. J Neurolin 2001; 16:999–1008.

18 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.

19 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.

20 Chere S, Miyashita H, Yamaguchi M, et al. Exploration of orally available calpain inhibitors: pepidyl alpha-ketoamides containing an amiphile at P9 site. Bioorg Med Chem 2001; 13:4473–4484.

21 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.

22 Han BH, Xu D, Choi J, et al. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxic-ischemic brain injury. J Biol Chem 2002; 277:30128–30136.
42 Hansson MJ, Mattiasson G, Mansson R, et al. The nonimmunosuppressive cyclosporin analogs NIM811 and UNIL025 display nanomolar potencies on permeability transition in brain-derived mitochondria. J Biomech Biomech 2004; 36:407–413.

43 Waldeymeier PC, Feldtrowl JJ, Cian T, Lemasters JJ. Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. Mol Pharmacol 2002; 62:22–29.

44 Marmarou CR, Povlishock JT. Administration of the immunophilin ligand cyclosporin A, FK506 and rapamycin on calcineurin phosphatase activity in mouse brain. JUBMB Life 2006; 58:429–433.

45 Yu DY, Luo J, Bu F, et al. Effects of cyclosporin A, FK506 and rapamycin on cerebral blood flow in rats. Exp Neurol 2006; 197:353–362.

46 O’Connor CA, Cernak I, Vink R. Both estrogen and progesterone attenuate edema formation following diffuse traumatic brain injury in rats. Brain Res 2005; 1062:171–174.

47 Dubal DB, Rau SW, Shughrue PJ, et al. Differential modulation of estrogen receptors in ischemic brain injury: a role for ER(alpha) in estradiol-mediated protection against delayed cell death. Endocrinology 2006; 147:3076–3084.

48 Roof RL, Hoffman SW, Stein DG. Progesterone protects against lipid peroxidation following traumatic brain injury in rats. Mol Chem Neurophatol 1997; 31:1–11.

49 Hoffman GE, Merchenthaler I, Zup SL. Neuroprotection by ovarian hormones in ischemic disease. Endocrine 2006; 29:217–232.

50 Brines ML, Ghezzi P, Keenan S, et al. Erythropoietin crosses the blood–brain barrier to protect against experimental brain injury. Proc Natl Acad Sci U S A 2000; 97:10526–10531.

51 Tsai PT, Ohah JJ, Kertesz N, et al. A critical role of erythropoietin receptor in neurogenesis and poststroke recovery. J Neurosci 2006; 26:1269–1274.

52 Sairainen T, Karjalainen-Lindsberg ML, Paetau A, et al. Apoptosis dominant in the perifrontal area of human ischaemic stroke – a possible target of antiapoptotic treatments. Brain 2006; 129:189–199.

53 Siren AL, Radyushkin K, Borelius S, et al. Global brain atrophy after unilateral panetal lesion and its prevention by erythropoietin. Brain 2006; 129:480–489.

54 This study monitored mice 3 and 9 months after lesioning, using high-resolution three-dimensional magnetic resonance imaging and behavioral testing; the same mice display global neurodegenerative changes. EPO, a hematopoietic growth factor, prevented behavioral abnormalities, cognitive dysfunction and brain atrophy when given for 2 weeks after acute brain injury.

55 Liu WG, Qiu WS, Zhang Y, et al. Effects of selective brain cooling in patients with severe traumatic brain injury: a preliminary study. J Int Med Res 2006; 34:58–64.

56 Seppelt I. Hypothermia does not improve outcome from traumatic brain injury. Crit Care Resusc 2005; 7:233–237.

57 Davies AR. Hypothermia improves outcome from traumatic brain injury. Crit Care Resusc 2005; 7:238–243.

58 Mcilvoy LH. The effect of hypothermia and hyperthermia on acute brain injury. AACN Clin Issues 2005; 16:486–500.

59 Donpenberg EM, Choi SC, Bullock R. Clinical trials in traumatic brain injury: lessons for the future. J Neurosurg Anesthesiol 2004; 16:87–94.

60 Wang KKW, Otten A, Liu MC, et al. Proteomic identification of biomarkers of traumatic brain injury. Expert Rev Proteomics 2005; 2:603–614.

This review summarizes the importance of discovering relevant TBI protein biomarkers and further outlines some of the desirable attributes of an ideal TBI biomarker.