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Themed Issue: Translational Neuropharmacology – Using Appropriate Animal Models to Guide Clinical Drug Development

REVIEW

Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here?

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Traumatic brain injury (TBI) is the leading cause of death and disability in young adults. Survivors of TBI frequently suffer from long-term personality changes and deficits in cognitive and motor performance, urgently calling for novel pharmacological treatment options. To date, all clinical trials evaluating neuroprotective compounds have failed in demonstrating clinical efficacy in cohorts of severely injured TBI patients. The purpose of the present review is to describe the utility of animal models of TBI for preclinical evaluation of pharmacological compounds. No single animal model can adequately mimic all aspects of human TBI owing to the heterogeneity of clinical TBI. To successfully develop compounds for clinical TBI, a thorough evaluation in several TBI models and injury severities is crucial. Additionally, brain pharmacokinetics and the time window must be carefully evaluated. Although the search for a single-compound, 'silver bullet' therapy is ongoing, a combination of drugs targeting various aspects of neuroprotection, neuroinflammation and regeneration may be needed. In summary, finding drugs and prove clinical efficacy in TBI is a major challenge ahead for the research community and the drug industry. For a successful translation of basic science knowledge to the clinic to occur we believe that a further refinement of animal models and functional outcome methods is important. In the clinical setting, improved patient classification, more homogenous patient cohorts in clinical trials, standardized treatment strategies, improved central nervous system drug delivery systems and monitoring of target drug levels and drug effects is warranted.

LINKED ARTICLES

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Abbreviations

aSDH, acute subdural haematoma; BBB, blood–brain barrier; CCI, controlled cortical impact; cFPI, midline (central) fluid percussion injury; CHI, closed head injury; CNS, central nervous system; CPP, cerebral perfusion pressure; CsA, cyclosporin A; CSF, cerebrospinal fluid; EDH, epidural haematoma; GCS, Glasgow Coma Scale; I/A, impact-acceleration; ICP, intracranial pressure; lFPI, lateral fluid percussion injury; NCC, neurocritical care; NMDA, N-methyl-D-aspartic acid; PBN, α-Phenyl-N-tert -butyl nitrone; rCBF, regional cerebral blood flow; S-PBN, 2-sulfophenyl-N-tert-butyl nitrone; TBI, traumatic brain injury; WDI, weight drop injury

Life brings on unexpected changes
But we must carry on despite it
– *Lenny Kravitz, the Future Song*

Introduction

Traumatic brain injury (TBI) caused by, for example, transportation, falls, assault, sports, mainly affects young individuals and is the leading cause of mortality and disability in the population below 50 years of age (Ghajar, 2000; Corrigan *et al.*, 2010). In Europe, TBI is the cause for 60 000 deaths annually and the incidence of fatal and hospitalized TBI combined was estimated to be 235/100 000 inhabitants. Although the global magnitude of TBI is unknown, available data suggest that the number of TBI victims globally is rising sharply (Tagliaferri *et al.*, 2006; Maas *et al.*, 2008; Corrigan *et al.*, 2010). The TBI frequently causes persistent functional deficits, leading to a loss of decades of productive life with a huge cost to the patient, his or her family and the society. Common consequences of TBI include personality changes, seizures, cognitive problems, impaired motor function and a reduced quality of life calling for long-term rehabilitation and treatment (Masel and DeWitt, 2010). A range of psychiatric disorders, where depression may be the most common, also occur after TBI (Bryant *et al.*, 2010). Currently, treatment of TBI according to established guidelines consists of supportive measures including the avoidance of prehospital hypoxia and hypotension, rapid surgery of mass lesions when needed, and neurocritical care (NCC) (Marion, 2006; Brain Trauma Foundation *et al.*, 2007). Improved NCC has been a major factor for improving outcome of severely brain-injured patients (Elf *et al.*, 2002). The outcome of TBI patients may also reflect the economic status of the region or country in which the patient is treated (Mauritz *et al.*, 2008). Further improvements in TBI outcomes may be achieved by a continued refinement of NCC based on research and development in the areas listed in Table 1. However, to date there are no specific neuroprotective pharmacological treatment options with proven clinical efficacy available for TBI patients (Maas *et al.*, 2008). Importantly, prolonged and supportive neurorehabilitation following the initial post-injury phase may also aid in improving outcome where a number of cognitive 'stimulants' have been evaluated to ameliorate post-injury disabilities (Napolitano *et al.*, 2005).

Traumatic brain injury may be the most complicated disease of the most complex organ of the body. This statement reflects the many features and distribution patterns of TBI as well as the sophisticated structure and function of the brain. For a successful translation of basic science knowledge to the clinic to occur in this context we believe that a further refinement of animal models and functional outcome methods as well as TBI patient classification and standardized treatment strategies is warranted (also see Morales *et al.*, 2005).

The purpose of this review is to discuss the current use of animal modelling of TBI as a tool in preclinical drug development with a focus on closed head injury (CHI) models in rodents, excluding the rapidly growing field of blast injury models.

Table 1

Key scientific approaches for further improvement of neurocritical care in traumatic brain injury

Pharmacological neuroprotection	Drugs blocking specific secondary injury mechanisms
	Drugs stimulating reparative mechanisms
Monitoring of the brain injury process	Biomarkers of specific secondary injury mechanisms
	Refined/novel neuroimaging methods
Neurorepair	Axonal regeneration
	Neurogenesis/stem cell therapy
	Neurorehabilitation/plasticity

Basic pathophysiology of TBI

The primary injury to the head causes rapid deformation of brain tissue with destruction of brain parenchyma and blood vessels causing damage to cell membranes with the immediate release of intracellular contents (McIntosh, 1994; Rink *et al.*, 1995). This initial injury cannot be treated, only prevented. Not all neuronal and glial damage occurs at the time of primary injury which is markedly exacerbated by a complex cascade of pathophysiological and neurochemical events during the course of the initial hours and days (Figure 1). Importantly, several reports have shown a progressive encephalomalacia for many years post-injury (Greenberg *et al.*, 2008; Ng *et al.*, 2008). Although the duration and magnitude of this secondary injury cascade may be highly variable among subtypes of TBI and among TBI patients, the concept of secondary brain injury is central to modern TBI management and the goal to improve outcome of TBI patients using novel pharmacological treatment options. A detailed overview of all aspects of the secondary injury cascade is beyond the scope of this review but key factors are highlighted below and in Figure 1.

In the immediate period following the primary injury, there is a massive disturbance of the cellular ion homeostasis initiated by excessive release of the excitatory amino acid neurotransmitters glutamate and aspartate with the subsequent activation of glutamate receptors, a process named excitotoxicity. The release of glutamate results in cellular influx of Na^+ and Ca^{2+} and efflux of K^+ (Faden *et al.*, 1989; Katayama *et al.*, 1990; Nilsson *et al.*, 1990; 1993). The influx of calcium ions is regarded to be a key event early post-TBI leading to mitochondrial damage, an increase in free radical production, changes in gene expression and activation of calcium-dependent proteases including caspases, calpains and phospholipases resulting in extensive cytoskeletal damage (Marklund *et al.*, 2001; Singleton *et al.*, 2001; Israelsson *et al.*, 2008). The marked mitochondrial perturbation post-TBI (Verweij *et al.*, 2000; Lifshitz *et al.*, 2004) leads to uncoupling of mitochondrial ATP synthesis at the time of increased energy demand due to activation of energy-consuming ion transport systems and cell repair enzymes.

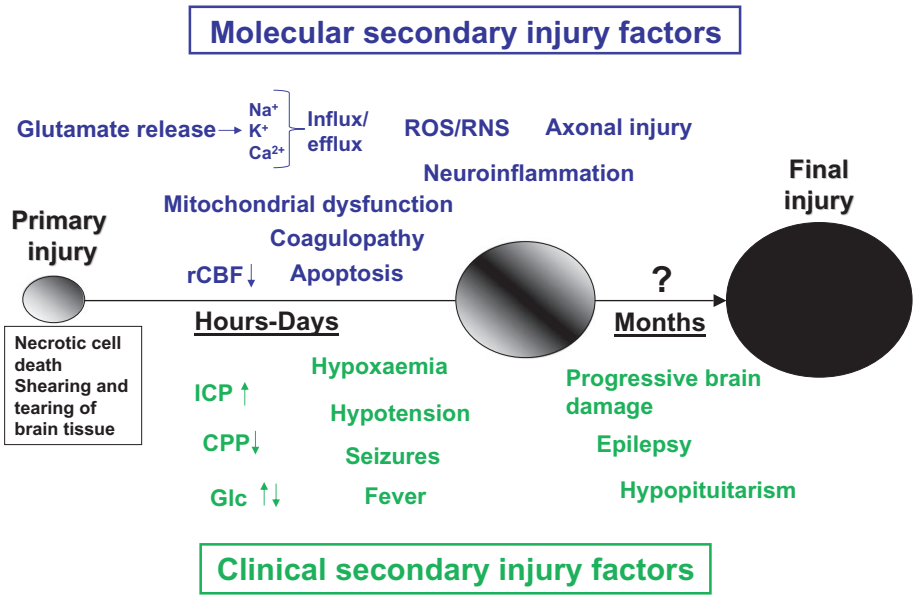

Figure 1

Basic concept of primary and secondary injury in traumatic brain injury. Simplistic illustration of major preclinical 'molecular' secondary injury factors as well as clinical secondary injury factors (named 'avoidable factors' in the neurocritical care setting). Early post-injury, glutamate release and ionic disturbances (Na^+, Ca^{2+} and K^+) cause an energy metabolic disturbance complicated by an early decrease in cerebral blood flow. At this time, mitochondrial disturbance is marked and a large increase in reactive oxygen/nitrogen species (ROS/RNS) is observed. Hyper- or hypocoagulation may also be present either causing microthrombosis or increased haemorrhages respectively. Neuroinflammation and axonal injury is emerging in the immediate post-injury phase. Clinically, an increased ICP and/or decreased CPP must urgently be treated and both too low and too high blood glucose levels corrected. It is also crucial that hypoxaemia and hypotension, seizures and fever is detected and treated. Chronically, marked hormone disturbance may be observed. These factors have been shown to contribute to the progression of the primary injury (indicated by the enlarging circles) and may be suitable targets for pharmacological intervention to reduce the extent of final injury. Ca^{2+}, calcium ions; CPP, cerebral perfusion pressure; GLc, Glucose; ICP, intracranial pressure; K^+, potassium; Na^+, sodium; rCBF, regional cerebral blood flow.

Conversely, an increase in glucose utilization or hyperglycolysis, has been observed across animal models and in humans early following TBI (Bergsneider *et al.*, 1997; Giri *et al.*, 2000). Unfortunately, the high demand for glucose and increase in local cerebral metabolic rate of glucose occurs at a time of reduced regional cerebral blood flow (rCBF) (Nilsson *et al.*, 1996; Ginsberg *et al.*, 1997; Marklund *et al.*, 2002) and this uncoupling of blood flow-cerebral metabolism may be deleterious to the injured brain post-TBI (Chen *et al.*, 2004). Increased oxidative stress is another key feature of the post-injury process because the brain is highly sensitive to free radicals or reactive oxygen/nitrogen species (ROS/RNS). Following TBI, there are several potential sources for overproduction of ROS/RNS, including the mitochondrial respiratory chain, increased free iron resulting from breakdown of extravasated haemoglobin, oxidation of catecholamines, breakdown of membrane phospholipids, NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) oxidase activation, infiltrating neutrophils and activation of nitric oxide synthetase occurring at a time when the intra- and extracellular antioxidant defence systems are challenged and may become exhausted (Shohami *et al.*, 1997; Hall *et al.*, 2010). Oxidative stress is currently thought to be a major contributor to the secondary injury cascade following TBI by ROS/RNS-induced damage to cellular membranes and organelles by lipid peroxidation, protein oxidation and nucleotide breakdown (Lewen *et al.*, 2000).

The TBI induces a robust immune activation including an acute inflammatory response with breakdown of the blood–brain barrier (BBB), oedema formation, infiltration of peripheral immune cells, activation of resident microglia and astrocytes and intrathecal release of cytokines (Schmidt *et al.*, 2005; Clausen *et al.*, 2009; Ziebell and Morganti-Kossmann, 2010). A prominent up-regulation of several chemokine-related gene transcripts was recently observed following focal TBI in mice (Israelsson *et al.*, 2008) and activated glial cells and leukocytes secrete a variety of neurotoxic molecules including tumour necrosis factor and the interleukin family of peptides (Adamchik *et al.*, 2000; Luheshi *et al.*, 2009; Lu *et al.*, 2009a). Importantly, infiltrating leukocytes secreting myeloperoxidase may be an important source for ROS by producing hypochlorous acid (see Lewen *et al.*, 2000). Conversely, numerous anti-inflammatory compounds have been evaluated in the experimental TBI setting and repeatedly shown to attenuate the behavioural and histological deficits post-TBI (Clausen *et al.*, 2009; Ziebell and Morganti-Kossmann, 2010). However, some inflammatory pathways may be important for regenerative responses and repair suggesting that inflammation may be a double-edged sword following TBI (Lenzlinger *et al.*, 2001; Morganti-Kossmann *et al.*, 2002; Whitney *et al.*, 2009).

Neural injury has been well documented in the first few hours after human TBI in important brain regions such as the cerebral cortex, hippocampus and thalamus and parts of the

brain stem (Kotapka *et al.*, 1994). The TBI-induced mitochondrial damage causes initiation of apoptotic cell death via an opening of the mitochondrial permeability transition pore, the release and activation of pro-apoptotic factors including soluble cytochrome c, apoptosis inducing factor and caspases (Lifshitz *et al.*, 2004; Mazzeo *et al.*, 2009a). It should be emphasized that TBI causes not only apoptotic but also necrotic neuronal cell death (Raghupathi, 2004). Regardless of the mechanisms for cell death, widespread neuronal damage occurs and may be observed even remote from the site of impact, where the hippocampal region may be particularly sensitive (Saatman *et al.*, 2006).

Although neuronal cell death has received the predominating attention in the TBI field it is obvious that traumatic axonal injury, often referred to as diffuse axonal injury (DAI), is common following TBI. Importantly, DAI is a dominant contributor to the functional deficits observed in TBI patients and is observed with high frequency in those unfortunate patients remaining in a persistent vegetative state (Graham *et al.*, 2005). Axonal injury is increasingly observed across injury severities and TBI subtypes due to the refinement of, for example, the magnetic resonance imaging technique (Inglese *et al.*, 2005). Acute axonal disconnection at the time of impact is rarely observed and only in patients with very severe TBI dying at the scene of the accident. Instead, stretching and shearing of axons caused by the impact may occur in areas remote to the impact and has been linked to the intra-axonal cytoskeleton damage that ultimately leads to axonal failure and disconnection (Ferrand-Drake *et al.*, 2003; Buki and Povlishock, 2006). Axonal swellings and axonal bulbs are the histological hallmarks of traumatic axonal injury, implying that axonal injury is an ongoing process that evolves over hours to days (Buki and Povlishock, 2006). When axons are disconnected, CNS axons do not spontaneously regrow following injury in contrast to axons in the peripheral nervous system. The reasons for the inability of CNS axons to regenerate are likely multifactorial although the most important factor may be the myelin-associated inhibitors of axonal growth including myelin-associated glycoprotein, oligodendrocyte-myelin glycoprotein and Nogo-A all binding to the neuronal Nogo-66 receptor (Sandvig *et al.*, 2004; Walmsley and Mir, 2007; Gonzenbach and Schwab, 2008). It should be remembered that unmyelinated axons comprise a numerical majority of fibres in subcortical white matter and these fibres may be particularly vulnerable to TBI (Reeves *et al.*, 2005).

Although damaged axons fail to regenerate, damaged neurons are not replaced and there is a marked glial scar counteracting any attempts for recovery, animals and patients often show a surprisingly high degree of spontaneous recovery over the initial months post-TBI. This spontaneous behavioural improvement is thought to partly reflect a remaining ability for neurophysiological and neuroanatomical changes in regions remote from a focal brain injury. Neuroanatomical plasticity, or the restructuring of neural connections in response to lesions of the CNS, is a well-documented phenomenon in the neonatal age group but highly restricted in adults. Nevertheless, an increased sprouting of uninjured corticospinal tract fibres into the injured tracts was observed 6 weeks following TBI in the rat (Lenzlinger *et al.*, 2005; Marklund *et al.*, 2007), suggesting that TBI may elicit yet unidentified mechanisms causing spontaneous sprouting that may be linked to the observed recovery post-injury. Additionally, increased expression of trophic factors as a part the inflammatory response, increased synapse formation and sprouting of hippocampal mossy fibres have all been observed following TBI although their role in the recovery process of TBI remains to be established (Scheff *et al.*, 2005; Hanell *et al.*, 2010). It should be emphasized that yet other factors, including disturbances in the neurotrophin, coagulation, endocrinological and neurotransmitter systems, may contribute to the pathology of TBI and be manipulated pharmacologically (Marklund *et al.*, 2006). In general terms, current pharmacological approaches may be roughly categorized into neuroprotection, inflammatory modulation and enhancement of regeneration.

Clinical TBI

Classification of human TBI is traditionally based on the symptoms and level of consciousness present on admission to hospital, generally using the Glasgow Coma Scale (GCS) and patients with severe TBI, that is, unconscious patients with a GCS score of ≤8, are frequently selected for severe TBI in clinical trials. Although the basic pathophysiology mentioned in the previous section may be common to most TBI patients, we strongly emphasize that TBI is not *one* disease (Figure 2). Instead, patients with similar clinical signs, symptoms and level of consciousness may have markedly different radiological appearance (including skull fractures, contusions, lacerations, axonal injury, BBB disruption, neurovascular injuries and haematoma with epidural, subdural, subarachnoid, intra-ventricular and/or intracerebral location; exemplified in Figure 2). Currently, acute treatment options for clinical TBI comprise optimal prehospital management and emergency room stabilization, surgery for space-occupying mass lesions, measurement and treatment of increased intracranial pressure (ICP) and the detection and treatment of secondary injury factors, for example, fever, seizures, hypoxia, hypotension (Figure 1) in a NCC setting (Elf *et al.*, 2002; Meyer *et al.*, 2010). The evidence-based guidelines for the treatment of TBI patients were recently published in revised form by the Brain Trauma Foundation *et al.* (2007). Pharmacological options in NCC include mannitol for reduction of emergent intracranial hypertension, sodium pentobarbital or other sedative drugs for reduction of brain metabolism and lowering of ICP in selected patients and antiepileptic drugs. However, none of these available compounds can be considered to have Class I evidence in the treatment of TBI. Due to the long-term and frequently life-long disabilities experienced by survivors of TBI (Masel and DeWitt, 2010), the development of novel pharmacological treatment options is of highest priority. Animal models of TBI are crucial in the preclinical drug development phase, reviewed in the next section.

Animal models of TBI

In view of the heterogeneous clinical situation, numerous TBI models have been developed. Mimicking all aspects of TBI in

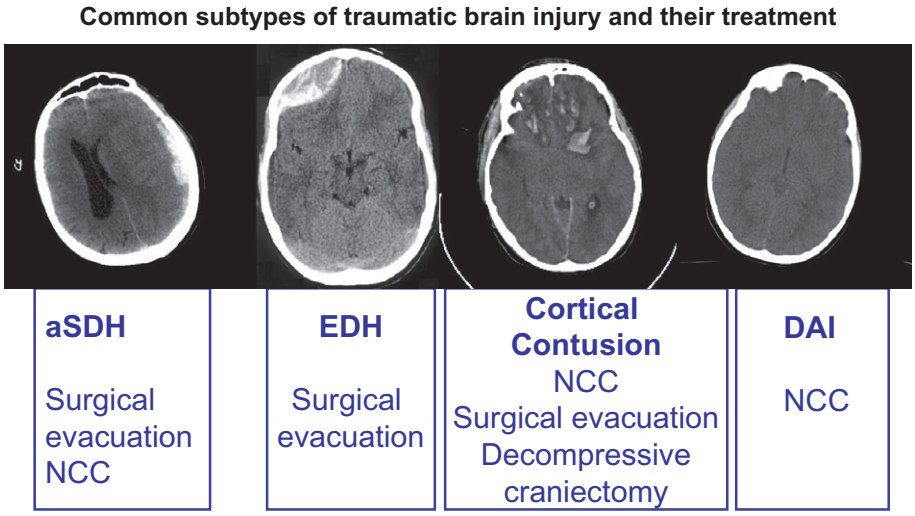

Figure 2

Key issue in clinical traumatic brain injury (TBI) research. TBI is not *one* disease as exemplified with initial computerized tomography scans of patients with severe TBI treated in our unit. These patients all had a decreased level of consciousness upon arrival in our unit. Typical primary treatment options for the individual TBI subtype are shown. aSDH, acute subdural haematoma; DAI, diffuse axonal injury; EDH, epidural haematoma; NCC, neurocritical care.

a single animal model is impossible and for that reason, a variety of TBI models are being used in animals of various ages and injury severity levels. Rodent models are the most common in TBI research due to their low cost and small size (Finnie and Blumbergs, 2002). In addition to the heterogeneity of TBI, the difficulty in evaluating subtle cognitive and psychiatric impairments in small animal species is a major challenge in the preclinical evaluation of neuroprotective drug candidates. Ideally, for an animal model to be useful in preclinical development of pharmacological compounds it needs to mimic the injury characteristics and severity observed in the clinical setting. Additional features of a useful preclinical TBI model are reproducibility, low costs, applicability to both rats and mice, technically easy to perform and, perhaps most important, production of long-lasting behavioural deficits (Morales *et al.*, 2005). Although even predominantly focal TBI shows a substantial degree of diffuse injury, we categorize the animal models in the next section into 'focal', 'mixed', 'diffuse', 'complex' and 'other' models of TBI for the sake of simplicity.

Focal TBI models

Controlled cortical impact. The most common brain lesion following human TBI is cortical contusion, defined as a focal destruction of brain tissue with micro haemorrhages, either with intact pia mater (contusion) or a torn pia mater (laceration). Importantly, contusions frequently enlarge markedly during the initial days post-TBI (Stein *et al.*, 1993). Cortical contusions predominantly occur in the frontal and temporal regions and are often observed also at brain regions opposite to the initial impact (contre-coup contusion). A common TBI model used to mimic this feature of principally focal human TBI is the controlled cortical impact (CCI; Figure 3) model, producing extensive cortical tissue loss, hippocampal and thalamic damage (Saatman *et al.*, 2006), cortical spreading depression (von Baumgarten *et al.*, 2008) and signs of post-traumatic epilepsy (Hunt *et al.*, 2009; Yang *et al.*, 2010). Using silver staining methodology, widespread ipsilateral but also contralateral axonal damage in the cortex, hippocampus and thalamus was also observed (Hall *et al.*, 2008). To induce CCI, a craniectomy over one hemisphere is performed to avoid skull fractures and brain injury is produced by a pneumatically driven rigid impactor striking the intact dura mater where the deformation of brain tissue including the time of compression, impactor velocity and depth of impact can be easily controlled using a computer-based monitoring device. Different designs of the impactor tip can also be used to vary the mechanical impact features. The CCI has been used in the ferret, sheep and swine, but rat (Dixon *et al.*, 1991) and mouse (Smith *et al.*, 1995) are currently the predominant species investigated with this model. Although CCI is rather fast and simple to use, reproducible and the injury severity can easily be adjusted, this TBI model also has some disadvantages. First, the contusion injury used in many reports is huge, frequently destroying the majority of the ipsilateral cortex and clearly not comparable with the extent of brain injury observed in survivors of human TBI. Second, a large craniectomy is often produced and if the bone flap is not replaced after injury, the effect of secondary brain swelling may be attenuated thus mimicking a decompressive craniectomy used for alleviation of raised ICP in humans (Zweckberger *et al.*, 2006; De Bonis *et al.*, 2010). Finally, the lack of brain stem damage in CCI will not produce long-lasting unconsciousness and carries a very low mortality, limiting the clinical relevance of this model. However, the rather low variability of the model, the reproducibility among centres and the long-lasting behavioural impairments still make the CCI model suitable for evaluation of pharmacological interventions.

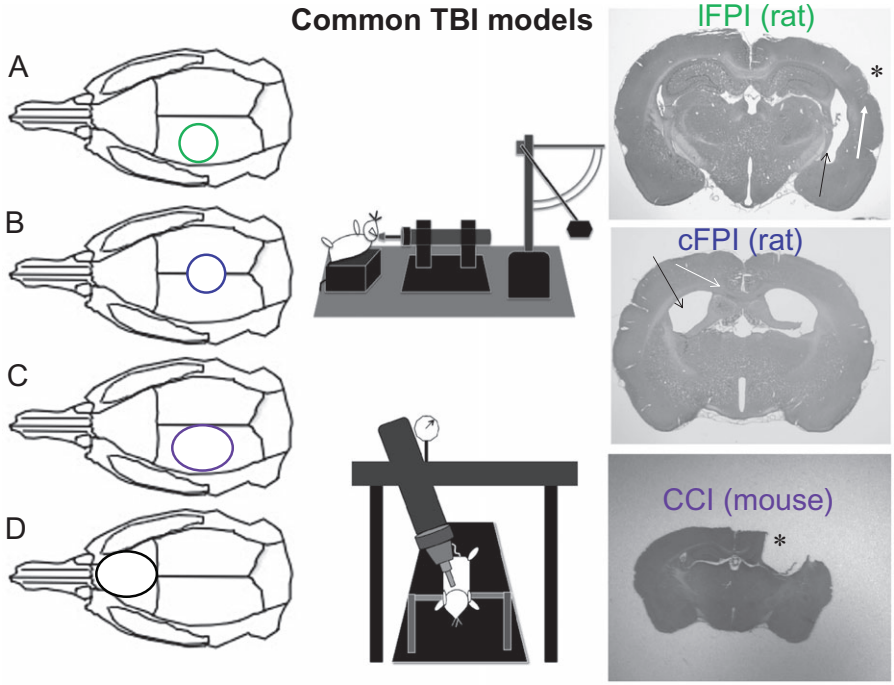

Figure 3

Examples of common preclinical traumatic brain injury (TBI) models mentioned in this review. Left panel: A = craniotomy position for lateral fluid percussion (lFPI) injury and B = craniotomy for the central/midline fluid percussion injury (cFPI). The fluid percussion device (top, middle panel) is used to produce the injury in both the lFPI and cFPI models (see text for details). In C and D, the controlled cortical impact (CCI) device is used (bottom, middle panel). In C, the craniotomy is placed over the parietal cortex and gives a chiefly focal contusion injury. In D, the craniotomy position is placed anterior to the bregma to produce a bifrontal contusion injury. Right panel; examples of the histological appearance from the models (A–C) from our institution. Upper right, examples from a mild lFPI injury in a rat (contusion site marked with * and the rather small cortical contusion with a white arrow). Note the ipsilateral ventricular dilatation (dark arrow). Right panel, middle image; example of a cFPI injury (rat). Note the bilateral ventricular dilatation (black arrow) and haemorrhages in the corpus callosum (white arrow). Right panel, lower image; example from a CCI brain injury in the mouse (impact site marked with *). Note the extensive cortical and hippocampal damage ipsilateral to the injury.

Weight drop injury. In the weight drop injury (WDI) model, an anaesthetized animal is subjected to TBI by a free-falling weight dropped from a predetermined height onto the exposed dura mater (open skull WDI) or the exposed skull (closed skull WDI). In the open skull WDI model the weight is dropped onto a piston resting on the exposed dura. Injury severity can be graded by varying the mass of the weight, the height or, in the open skull WDI model, by varying the depth of compression by different designs of the piston. To minimize variability the head is fixed in a restraint to prevent movement. Frequently, the device has the measures to prevent bouncing of the weight which may be difficult to control. The WDI model is used in the rat using an open skull technique and in both the mouse and rat using a closed skull technique (Chen *et al.*, 1996; Henninger *et al.*, 2005; Morales *et al.*, 2005; Flierl *et al.*, 2009) where a skull fracture may be needed to produce a cortical contusion (Flierl *et al.*, 2009).

The open skull WDI model shows several similarities to the CCI model, producing a chiefly focal brain injury with a cortical contusion and ipsilateral hippocampal and thalamic damage, although some bilateral components to this injury exist (Lewen *et al.*, 1996; Marklund *et al.*, 2001; Clausen *et al.*, 2005). Important information on the neurochemistry of TBI and the comparison between clinical and rodent TBI has been achieved using cerebral microdialysis in this model (Katayama *et al.*, 1990; Nilsson *et al.*, 1990), including post-traumatic seizure activity (Nilsson *et al.*, 1994). Because the vast majority of clinical TBI is CHI, concerns were raised to the clinical validity of the open WDI (Flierl *et al.*, 2009). On the other hand, when using the closed WDI model the incidence of skull fractures increases with rising injury severity and may also cause a substantial degree of diffuse injury (*vide infra*). As an example, a cerebral concussion, clearly a diffuse type of TBI was simulated in a rat closed WDI model using head fixation (Henninger *et al.*, 2005).

In all, the WDI models are fast and reliable and produce a significant degree of brain damage, neuroinflammation and behavioural deficits including cognitive impairment and provide important information on focal TBI in both rats and mice.

Bifrontal contusion. Contusions commonly occur in the frontal region and are an important contributor to the observed personality changes following human TBI (Fork *et al.*, 2005). Due to the clinical importance of this injury type, a bifrontal contusion rat model was developed by Dr Stein's group that showed histological damage in the medial prefrontal cortex and impairment in cognitive and motor function (Hoffman *et al.*, 1994). This model may be considered a modification of the CCI model and following the

central craniectomy the impactor is placed over the midline centred anterior to bregma. This model represent a preclinical approach to an important clinical problem and has been repeatedly used in numerous pharmacological studies (Goss *et al.*, 2003; Djebaili *et al.*, 2005), particularly on endocrinological treatment options. The ongoing progesterone clinical trial (*vide infra*) is to a large extent based on preclinical data obtained in this model.

Acute subdural haematoma. Acute subdural haematoma (aSDH) is a major cause for the acute mortality observed following clinical TBI. Improved outcome was clearly demonstrated if surgery was initiated within 4 h after the injury suggesting that time is crucial in the management of aSDH (Seelig *et al.*, 1981). Following surgery, many patients still need NCC owing to persisting depression of the level of consciousness, focal deficits and cerebral swelling. There are rodent models of aSDH developed for both the rat and mouse in which autologous blood is injected into the subdural space. Common findings include uncoupling of metabolism/blood flow, development of ischaemia and, for example, dextromethorphan, sodium channel antagonists and glutamate receptor antagonists have been evaluated using these models (Miller *et al.*, 1990; Tsuchida and Bullock, 1995; Tsuchida *et al.*, 1996; Wang *et al.*, 2010). A model of aSDH in the mouse has also been developed (Sasaki and Dunn, 2001). To date, only a minority of TBI studies in general and pharmacological treatments in particular are conducted in these models despite the huge clinical importance of aSDH.

Epidural haematoma. Epidural haematoma (EDH) is commonly associated with skull fractures and is caused by either arterial or venous haemorrhage or a combination of both. In the clinical setting, by far the most important treatment is rapid surgery which is indicated for the evacuation of space-occupying haematomas. The brain compression caused by the EDH may cause secondary oedema that may require NCC and ICP monitoring. To date, the mortality of EDH is still high. Only rarely used animal models of EDH employed in rats, dogs and pigs exist where EDH is simulated by either an inflated balloon or injection of autologous blood into the epidural space (Ganz *et al.*, 1990; Ebmeyer *et al.*, 1998; Balikci *et al.*, 2008). Pharmacological treatment has currently no role in the management of EDH in the clinical setting although vitamin E-supplemented rats showed increased blood flow in the compressed cortex following EDH evacuation (Busto *et al.*, 1984).

Mixed TBI models

The lateral fluid percussion injury (lFPI) model is an important TBI model used worldwide following its introduction by McIntosh *et al.* (1989), reviewed by Thompson *et al.* (2005; see Figure 3). In this model, the skull is exposed and a craniotomy is performed, typically over the parietal cortex, and a plastic cap is secured over the craniotomy using dental cement. Injury is produced by the release of a pendulum, hitting the end of a saline-filled reservoir producing a pressure wave creating a fluid bolus which strikes the intact dural surface to extend into the epidural space causing brain deformation (Sullivan *et al.*, 1976). In humans, the duration of the force creating brain dislocation after falls is estimated to be approximately 25 ms (Lindgren, 1966) which is similar to the pressure wave produced by lFPI. Although mostly used in rats, the lFPI model has also been adapted to the mouse (Carbonell *et al.*, 1998). Importantly, the severity of the injury produced by the lFPI model may easily be varied to evaluate also aspects of mild TBI in both mice (Spain *et al.*, 2010) and rats (Li *et al.*, 2006). At the time of injury, a short-lasting apnoea and concomitant seizures are frequently observed and seizures are common at long-term post-injury (D'Ambrosio *et al.*, 2004; Kharatishvili *et al.*, 2006; Kharatishvili and Pitkanen, 2010). The lFPI model produces consistent behavioural (cognitive, motor, complex) deficits and necrotic and apoptotic cell death in the cortex, hippocampus and thalamus (Thompson *et al.*, 2005). In addition to a focal cortical contusion ipsilateral to the impact, there is also a significant degree of axonal injury in the capsula interna and externa and the corpus callosum making this injury model clinically relevant (Graham *et al.*, 2000). Limitations of the lFPI model include some variability and it may be, at least initially, technically challenging with marked differences in outcome among technicians and centres at similar injury severity levels. Even minor changes in craniotomy position may result in large differences in histological and behavioural outcome (Floyd *et al.*, 2002). One important shortcoming of this model, similar to the focal TBI models, is the lack of long-lasting coma and brain stem damage. However, the lFPI model remains very important in the preclinical evaluation of drug candidates.

Diffuse TBI models

A large proportion of patients suffer from diffuse TBI, evidenced by traumatic subarachnoid haemorrhage, diffuse oedema, small intraparenchymal haemorrhages and DAI (Adams, 1982). In fact, the majority of TBI patients have at least a degree of diffuse injury (Maas *et al.*, 2007). Predominant locations for DAI are the parasagittal white matter, corpus callosum, internal capsule, the thalami, and parts of the brainstem and ventricular dilatation is commonly observed (Ai *et al.*, 2007). Mechanistically, angular acceleration/rotation and, to an uncertain extent, linear acceleration is the primary cause of axonal injury (Smith *et al.*, 2003; Fijalkowski *et al.*, 2009). Some diffuse TBI models produce a degree of angular acceleration without an associated impact and inertial acceleration injury models have been developed using the non-human primate, pig, sheep, cats and rabbits (see Morales *et al.*, 2005). These models produce clinically relevant damage to deep white matter tracts and grey-white matter junction sliding contusions but their use in pharmacological studies remain to be established. Because rotational acceleration forces are required to induce axonal damage, rotational models have also been developed for the rat (Xiao-Sheng *et al.*, 2000; Ellingson *et al.*, 2005; Fijalkowski *et al.*, 2007; Kilbourne *et al.*, 2009; Li *et al.*, 2010). Although these modifications of diffuse TBI models have many theoretical advantages, they still need to be defined in terms of long-term behavioural deficits and more extensive pathophysiological evaluation in order to be used as tools for pharmacological evaluation. To date, the impact/acceleration (I/A), midline (central) fluid percussion injury (cFPI) and what we refer to as the 'CCI-based' models are the most

Table 2

Magnesium and cyclosporin A evaluation in rodent TBI models

Drug	TBI model	Route of administration	Time window	Repeated administration	Outcome measure	Time for outcome
Magnesium	lFPI[1–6,13–14,16,22] I/A[7–11,15,17,19–20] CHI[12] Bifrontal contusion[18] CCI[23,24,27,28] Rat[1–22] Mouse	i.v.[1–7,9–11,13–16,18,19,22] i.p.[17,20,21] s.c.[12] i.m.[8,10]	Pre-injury[3,22] ≤1 h[1,2,4–7,9–21] 1–6 h[8] >6 h[8]	Yes[8,18] No[1–7,9–17,19–22]	Cognition[1,2,11,14,16,18,19,22] Motor/ Sensorymotor[2–4,6–12,14,18,19,21] Oedema[2,17,20] Lesion vol/cell death[5,6,13,16,19,22] Complex[11,16]	≤7 days[1,6,8–10,12,13,17,18,20] 7–28 days[2–5,11,14,19,21,22] >28 days[15,16]
Cyclosporin	lFPI[25,26,28] CCI[23,24,27,28] Rat[25–28] Mouse[23,24]	i.v.[25,27] i.p.[23,26,28] s.c.[24] i.m.	Pre-injury[23,25] ≤1 h[23,24,26,27] 1–6 h[28] >6 h[28]	Yes[23–26,28]* No[27]	Cognition[25,26] Motor/ Sensorymotor[25,26] Oedema[27] Lesion vol/cell death[23,24,28] Complex	≤7 days[23,24,27,28] 7–28 days[25,26] >28 days

References to Table 2:
[1]Smith *et al.* (1993), [2]Okiyama *et al.* (1995), [3]McIntosh *et al.* (1988), [4]McIntosh *et al.* (1989), [5]Bareyre *et al.* (2000), [6]Guluma *et al.* (1999), [7]Heath and Vink (1998), [8]Heath and Vink (1999b), [9]Heath and Vink (1997), [10]Heath and Vink (1999c), [11]Vink *et al.* (2003), [12]Feldman *et al.* (1996), [13]Saatman *et al.* (2001), [14]Bareyre *et al.* (1999), [15]Fromm *et al.* (2004), [16]Browne *et al.* (2004), [17]Esen *et al.* (2003), [18]Hoane (2005), [19]Turner *et al.* (2004), [20]Imer *et al.* (2009), [21]Barbre and Hoane (2006), [22]Enomoto *et al.* (2005), [23]Scheff and Sullivan (1999), [24]Sullivan *et al.* (2000a), [25]Alessandri *et al.* (2002), [26]Riess *et al.* (2001), [27]Fukui *et al.* (2003), [28]Sullivan *et al.* (2000b).
Overview of preclinical studies evaluating Mg^{2+} or cyclosporin A in rodent models of TBI.
*Prolonged, >1 h infusion, included in the Repeated administration column.
CCI, controlled cortical contusion injury; CHI, closed head injury; i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; I/A, impact-acceleration; lFPI, lateral fluid percussion injury; s.c., subcutaneous; TBI, traumatic brain injury.

commonly used diffuse TBI models as discussed in the following section.

The I/A ('Marmarou') model. Since its introduction, the I/A weight drop model (Foda and Marmarou, 1994; Marmarou *et al.*, 1994), commonly called the 'Marmarou model', has become a widely used diffuse head injury model in rat. The trauma device used in this model consists of a weight falling from a designated height through a tube onto a stainless steel disc glued to the skull of the rat. The head of the anaesthetized rat rests on a platform covered by a foam with a carefully defined 'spring constant' to allow a controlled movement of the head after impact. The model may be considered a high-level weight drop model where the use of the steel disc distributes the energy diffusely over the brain, and the foam enables dorsal/ventral acceleration of the unrestrained head. With this relatively easy to perform model, widespread axonal injury is observed and, importantly, this model is one of few TBI models that can result in prolonged unconsciousness. Behavioural deficits have been reported after I/A TBI in the rat up to 1 month post-injury (Beaumont *et al.*, 1999; Cernak *et al.*, 2001; Rancan *et al.*, 2001; O'Connor *et al.*, 2003). One limitation of the Marmarou model is that it is unavailable for mice, although a similar technique of a weight dropped on a disc was used in mice (Kupina *et al.*, 2001). The Marmarou model has provided much useful information on the pathobiology of DAI and results in an injury similar to what is observed in humans. Although pharmacological studies have been conducted in this model, mainly from the lab of Drs Povlishock and Vink (see Table 2; Buki *et al.*, 2003; Thornton *et al.*, 2006; O'Connor *et al.*, 2007; Harford-Wright *et al.*, 2010), its use in such experiments is still rather scarce particularly in terms of behavioural outcome. Thus, this model could and should be used more frequently for long-term pharmacological evaluation.

Midline (central) fluid percussion injury. Originally used in the cat and rabbit and later adapted to pigs, dogs and sheep, the popularity of the cFPI model increased when it was modified for use in the rat (Dixon *et al.*, 1987; McIntosh *et al.*, 1987). The model uses a similar set-up as the lateral fluid percussion (*vide supra*) with the exception that the craniotomy and cap placement is centred at the midline between the bregma and lambda sutures and over the sagittal sinus (Figure 3). In addition to the production of widespread axonal injury and hippocampal damage with accompanying brain stem damage, persistent motor and cognitive impairment has been demonstrated (Morales *et al.*, 2005). The cFPI model produces a clinically relevant diffuse brain injury with TBI-induced hypertension, increased ICP and a reduced cerebral blood flow (Kabadi *et al.*, 2010). An additional strength of this model is the recent murine application (the lab of Dr J. Povlishock at the Medical College of Virginia, pers. comm.; as well as our own lab, N. Marklund and L. Hillered, unpubl. results). Shortcomings of the cFPI model include a rather challenging and time consuming surgical preparation and that only a limited injury severity can be achieved because

of acute mortality owing to brain stem damage. Although there are numerous pharmacological studies using the cFPI model, particularly on cognitive function, more long-term evaluation of the behavioural consequences and treatment efficacy is warranted.

Diffuse TBI models using a pneumatically driven device, 'CCI-based' models. The CCI device used to produce a focal TBI and described in a previous section of this review, may also be modified to create a diffuse injury by placing the impact over the midline (Lighthall, 1988) or using bilateral craniotomies (Meaney *et al.*, 1994). However, other similar models have been introduced and achieved a higher popularity. Common for these models is that the pneumatically driven impactor strikes a steel disc fixed on the skull of the animal that rests on a gel-filled base allowing for some movement of the head at impact. These models have been used in both rats and mice (Cernak *et al.*, 2004; Laskowitz *et al.*, 2007; Maruichi *et al.*, 2009). Advantages of this setup include the ease and speed of the surgical preparation and the addition of the steel disc enables the impact energy to be distributed diffusely over the brain tissue resulting in hippocampal, brain stem and axonal damage with motor and cognitive deficits. These models have some impact variability and the relevance to the human situation needs to be better defined. However, these models have been successfully used in preclinical pharmacological research and represent an interesting addition to the 'classical' TBI models.

In conclusion, diffuse injury models produce a significant degree of axonal injury, of highest importance due to the vast clinical problem of DAI, and should in our opinion be available in most labs evaluating the efficacy of pharmacological compounds for TBI.

Complex TBI models

It has been demonstrated beyond doubt that additional, concomitant systemic injuries markedly exacerbate the outcome following TBI. Hypoxia (PaO_2 < 60 mmHg) and, in particular, hypotension (systolic blood pressure <90 mmHg) are the most common and occur in about one third of patients with severe TBI (Chesnut *et al.*, 1993). A common notion has been that animals are far more resistant to secondary insults than humans (Lammie *et al.*, 1999). However, other studies have demonstrated an impaired motor and histological outcome in animals subjected to TBI and hypoxia (Ishige *et al.*, 1987; Clark *et al.*, 1997) and the time period between the injury and the secondary insults may be highly important (Geeraerts *et al.*, 2008). Thus, the CCI and I/A models have been used in combination with hypoxia (Hellewell *et al.*, 2010), hypoxia and hypotension (Robertson *et al.*, 2000; Stiefel *et al.*, 2005; Taya *et al.*, 2010) and the lFPI model with hypoxia (Bramlett *et al.*, 1999; Bauman *et al.*, 2000) and/or hypotension (Matsushita *et al.*, 2001; Aoyama *et al.*, 2008). The secondary insults were shown to exacerbate histological and behavioural outcome in these studies and were also implemented in a model of aSDH (Sawauchi *et al.*, 2004). In all, these models represent an excellent example of a true translational approach where an important clinical problem is addressed in the experimental setting. One elegant additional model is the combination of the lFPI model with a tibial fracture (Maegele *et al.*, 2005; Maegele *et al.*, 2007). The effects of hypothermia have been evaluated in these models (e.g. Robertson *et al.*, 2000; Gao *et al.*, 2010) although pharmacological intervention studies are scarce. We suggest that pharmacological treatment options be evaluated also in the combined TBI models prior to launching full-scale clinical trials enrolling patients with multiple injuries.

Other TBI models

The use of larger animals such as the pig or piglet in TBI research is increasing (Smith *et al.*, 1999; Missios *et al.*, 2009) and examples of such TBI models being used in pharmacological evaluation is emerging (e.g. Zhang *et al.*, 2008; Armstead *et al.*, 2010). These TBI models may gain wider use than those using non-human primates and may hopefully be more commonly employed for pharmacological evaluation in the future. Although outside the primary scope of this review, additional TBI models only rarely evaluated in pharmacological studies are briefly mentioned in the following section.

Repetitive models. Epidemiological data suggest an increased risk for onset of neurodegenerative diseases, such as Alzheimer's disease, in people who has suffered repetitive head injury. In addition, repeated mild TBI commonly observed in athletes (soccer, boxing, ice hockey, etc.) may have cumulative adverse effects on cognitive function. Several models aim at reproducing the clinical consequences of repetitive mild TBI (see Morales *et al.*, 2005). Repetitive concussive brain injury models have also been developed for use in the mouse using the weight drop model (DeFord *et al.*, 2002; Creeley *et al.*, 2004) and in the rat using repeated, up to four, mild concussive insults using the lFPI model (DeRoss *et al.*, 2002). For instance, using the CCI device modified using a rounded silicone impactor on the intact skull in the mouse, the animals were subjected to a mild injury and then to a second injury at various intervals post-injury. Results from these studies showed that a second injury impaired motor and histological outcome with increased axonal pathology if the second injury occurs within a 3- to 5-day period of the first (Laurer *et al.*, 2001; Longhi *et al.*, 2005). Models of repetitive TBI have also been used in mice over-expressing human Amyloid precursor protein (Tg2576 mice), in which vitamin E treatment resulted in less amyloidosis and improved cognitive function (Conte *et al.*, 2004). Although these models provide insight into the field of repeated head injury of value for, for example, athletes at risk for repeated concussions, pharmacological evaluation has only rarely been performed.

Blast injury models. Rapidly growing field of interest is based on the recent experience from combat field activities in Iraq and Afghanistan where an increasing number of soldiers exposed to blast waves from detonations suffer functional sequelae requiring long periods of treatment. Rodent models of blast TBI have recently been established (Cheng *et al.*, 2010; Risling *et al.*, 2011; Saljo *et al.*, 2010; Svetlov *et al.*, 2010). However, to our knowledge, no post-injury pharmacological intervention studies have been conducted to date and long-term behavioural and morphological consequences need to be established. These models of blast injury are still at their infancy although future studies will likely provide novel

information on the mechanisms of, for example, axonal injury and its treatment.

Penetrating injury models. A model of penetrating brain injury has been characterized in the rat (Williams *et al.*, 2005; Williams *et al.*, 2006a,b) and shown to produce cognitive impairment (Davis *et al.*, 2010). In this model, a metal rod covered with an elastic tube that rapidly (10 ms) inflates and deflates is used to produce a shock wave along the injury tract to mimic a penetrating ballistic injury. Marked grey and white tissue damage, brain swelling, seizures, cortical spreading depression and neuroinflammation with a resulting neurological impairment was demonstrated. This model has been recently used in pharmacological evaluation (Shear *et al.*, 2009; Lu *et al.*, 2009b) and future studies are needed to define the role of this model in the development of neuroprotective compounds.

Although these models have not, or rarely, been used in pharmacological studies, it is important to emphasize that treatment efficacy of a compound in one TBI model does not in any way guarantee efficacy in other TBI models. Thus, more studies of efficacy of treatment compounds across a range of TBI models are needed.

Outcome measures

Following human TBI, long-term changes in personality, cognitive performance and motor function is common and leads to a marked reduction in the quality of life. Spontaneous recovery is most pronounced within the first 6 months after the TBI but may vary with different types of TBI (Consensus conference, 1999). Usually, outcome is assessed in the clinical situation at 3 or, more commonly, 6 months post-injury using increasingly complex batteries of neuropsychological tests (Bagiella *et al.*, 2010). However, the Glasgow Outcome Scale (or its extended version GOSe) remains a basic outcome measure included in the majority of clinical trials (Maas *et al.*, 2008). In the experimental setting, there are numerous behavioural tests in use where the evaluation periods are relatively short and only rarely extending beyond 1 month post-injury (Marklund *et al.*, 2006). To date, cognitive and motor function tests are routinely included in outcome evaluations (Fujimoto *et al.*, 2004). Widely used tests for cognition include the Morris water maze, object recognition test, memory task, freezing response and others. Common motor function tests include the Rotarod, cylinder test, skilled forelimb reach, grip strength tests and staircase tests. In view of the complex personality and psychological disturbances experienced by many TBI patients, more complex behavioural assessment test have also been used in experimental TBI research, albeit less frequently. These tests include the open field, elevated plus maze, spontaneous motor activity, exploratory activity and emotional activity tests. In addition, using lFPI in rats, a pervasive hyperanxious phenotype was observed (Jones *et al.*, 2008). Depression is also a major clinical problem post-TBI that has not been thoroughly studied in animal models although there are reports using the Porsolt test of forced swimming (Tweedie *et al.*, 2007). There is concern that the outcome measures in clinical trials and the behavioural tests used in preclinical research are not well matched (Fujimoto *et al.*, 2004) calling for a continual refinement of experimental outcome methods. As an example, a novel behavioural model for testing numerous spontaneous complex tasks was recently established for TBI in mice (Ekmark-Lewen *et al.*, 2010) using a test situation with a free choice of different optional environmental settings that may be useful for behavioural evaluation in pharmacological research. Although it is obvious that evaluation of rodents cannot directly be compared to that of humans, scientists evaluating pharmacological compounds are encouraged to incorporate more complex behavioural assessment tools in addition to standard motor and cognitive tests into the study protocols.

Neuroprotective pharmacology in TBI – why no success thus far?

As outlined in the short overview in the previous sections, there is a plethora of TBI models and outcome measures used in many institutions worldwide, frequently employed for evaluation of neuropharmacological interventions. In our opinion, the key to successful preclinical development of pharmacological compounds for the future lies not within the development of yet more TBI models, but instead that existing animal models be modified to better mimic the complex clinical situation. It is also evident that features of TBI observed in the clinical setting should be added to existing TBI models to enhance clinical applicability. Although some previous clinical trials in TBI have been conducted based on preclinical efficacy only in stroke models it should be emphasized that TBI and ischemic stroke, although sharing some common mechanisms, are truly two very different diseases of the CNS (Bramlett and Dietrich, 2004). It appears logical that clinical and experimental TBI research work together in a translational fashion in order to evaluate experimental findings clinically and vice versa, and that clinical experience and knowledge be incorporated into preclinical TBI research. In the treatment of TBI there are numerous pharmacological aspects to consider; for example, treatment dose and time window, target brain concentration, route of administration and efficacy in modifying certain aspects of the pathophysiology of TBI. Most drug companies and scientist focus on the development of a single, 'silver-bullet' therapy although a more realistic approach may be a combination of compounds using different targets (Margulies and Hicks, 2009; Loane and Faden, 2010). In fact, several compounds such as minocycline, erythropoietin and 2-sulfophenyl-N-tert-butyl nitrone (S-PBN), have been shown to act as multifunctional therapeutics influencing multiple targets in the secondary injury cascade. In the next section, we outline some common shortcomings and problems of existing TBI models and provide suggestions on how to further improve these models.

Time window, route of administration and the blood–brain barrier

In the vast majority of preclinical protocols, the treatment compounds are administered early and, frequently, even

prior to the TBI. The administration of a compound early by prehospital care personnel may be problematic because of the difficulty in obtaining informed consent (Menon, 2009). For that reason, the time allowed for drug administration in clinical trials has often been extended way beyond the time window suggested by preclinical trials. In fact, time window issues may be a major cause of the failure of the randomized clinical trials evaluating neuroprotective compounds. More research on extended time windows in the experimental setting is needed. Specifically, the temporal appearance of the secondary injury mechanism at target must be studied in detail. Furthermore, numerous experimental studies have employed oral, intraperitoneal or intracerebroventricular administration routes that may have restricted clinical utility. Additionally, single-dose treatment using high drug doses has commonly been employed experimentally, in contrast to the clinical setting where continuous infusion of a lower treatment dose, in order to avoid adverse side effects, is standard. In the NCC setting, numerous drugs, such as pentobarbital and phenytoin, are used which may cause drug interaction problems and influence clearance and distribution volume of the study drugs (Empey *et al.*, 2006). In addition, altered cerebral metabolism, changed gene expression and protein synthesis may influence the clearance, brain penetration and distribution of a compound (see Boucher and Hanes, 1998; Lo *et al.*, 2001; Kalsotra *et al.*, 2003). Thus, a correct translation of dosage of a compound from the experimental to the clinical setting requires careful pharmacokinetic evaluation in humans.

In terms of pharmacology of TBI, the BBB must be considered. In fact, knowledge about in which patient and at what time the BBB is open may be key factor in future clinical trials (Blyth *et al.*, 2009). The BBB is a physical barrier, composed of capillary endothelial cells connected by tight junctions together with astrocytes, that actively controls the penetration of molecules and microscopic objects from the blood into the brain. Small hydrophobic molecules (e.g. O_2, CO_2, anaesthetics, barbiturates, ethanol and hormones) and water readily pass through the BBB whereas diffusion of microscopic objects (e.g. bacteria), large molecules (e.g. plasma proteins) or hydrophilic molecules (e.g. electrolyte ions) is restricted. Metabolic products such as glucose and lactate, pyruvate are actively transported across the BBB by specific proteins (Glucose transporter 1 and monocarboxylate transporters respectively). In numerous studies, TBI has been shown to alter the status of the BBB allowing for the passage of substances normally restricted to the blood stream. The duration of this BBB opening in TBI models is variable but typically exists for a minimum of 3–7 days and the BBB disturbance may appear in certain brain regions only and with a different temporal profile for different molecular weight substances (Cortez *et al.*, 1989; Habgood *et al.*, 2007; Kelley *et al.*, 2007). Due to an altered BBB, pharmacological compounds hindered from entering the normal brain may thus reach the injured brain. To take advantage of this situation in the NCC setting novel tools for monitoring BBB function in TBI patients is urgently needed. The possibility that TBI-induced oedema and reduced blood flow may influence drug transport into the injured brain should also be considered. Ongoing research using chemically modified pharmacological compounds (Banks, 2008) with improved brain penetration may be an additional method for the treatment of TBI.

Thus, knowledge of brain penetration and pharmacokinetics is highly relevant although only rarely evaluated in preclinical research. In fact, most neuroprotective trials have been conducted without such preclinical documentation. We would like to encourage scientist to measure the penetration of the study compound into target brain regions. In this context, monitoring of a suitable surrogate end point biomarker by cerebral microdialysis may enable evaluation of the appropriate therapeutic window, dosage and brain penetration of the study compound also in clinical TBI research. Another key issue is to establish whether a drug has the desired effect on a specified mechanism *in vivo*. Again, such questions may be addressed using microdialysis to monitor a suitable surrogate end point biomarker in TBI patients. In a previous report, the free interstitial concentration of the glutamate release inhibitor Topiramate as well as glutamate was measured and a significant lowering of interstitial glutamate was achieved only after the Topiramate dose was elevated to a dose much higher than anticipated at the planning stage (Alves *et al.*, 2003). These data strongly support the importance of determining adequate target drug concentration and proof-of-principle testing before moving to large scale clinical trials (Alves *et al.*, 2003; Helmy *et al.*, 2007). The proof-of-principle approach also helps to optimize the timing of drug administration in relation to the time course of the injury mechanism at target.

Finally, it should be noted that it may not be mandatory for all drugs to penetrate the BBB to exert a neuroprotective effect. Important information was obtained when the brain penetration of two ROS scavengers, the nitrone spin traps S-PBN and α-Phenyl-N-tert -butyl nitrone (PBN), were compared. Although S-PBN did not reach the injured brain in detectable amounts in contrast to PBN, equal ROS scavenging properties and behavioural outcome improvement were obtained suggesting that brain penetration was not an absolute requirement for efficacy (Marklund *et al.*, 2001). This notion is also supported by the preclinical information obtained using tirilazad and polyethylene glycol-conjugated superoxide dismutase both without significant brain penetration but with neuroprotective properties (see Marklund *et al.*, 2006). These results suggest that the cerebral microvascular endothelium may be an important target for pharmacological intervention following TBI.

Animal models of TBI – are they good enough?

One crucial question is – do current experimental TBI models adequately mimic clinical head injury? Rodents are vastly different from humans in terms of brain size and geometry, white-to-grey matter ratio and they lack the cortical gyri observed in higher species (Gennarelli, 1994). Major clinical problems such as emotional and language difficulties are obviously not possible to mimic using rodent models. There is also a lack of TBI models producing long-lasting periods with a decreased level of consciousness or coma, commonly observed in severely injured TBI patients. Most laboratories

also typically employ TBI models that, in reality, represent injuries on the mild-moderate scale, in sharp contrast to the severely brain-injured patients evaluated in clinical trials (see Thompson *et al.*, 2005). Another treatment strategy for severe clinical TBI that is increasingly recognized is decompressive craniotomy (Piek, 2002), which may prove difficult to evaluate in rodent models due to differences in brain conformation and anatomy, degree of cerebral swelling and skull anatomy. In contrast, animal models appear to adequately mimic certain aspects of blood flow-energy metabolic disturbances post-injury. In patients dying from severe TBI, 90% were found to have ischemic lesions upon autopsy (Graham *et al.*, 1978; Kotapka *et al.*, 1994). However, observations in TBI patients in the NCC setting suggest that ischaemia may be a less prominent feature in TBI than previously thought and, instead, other types of energy metabolic perturbations unrelated to hypoxia/ischaemia appear to be common (Hlatky *et al.*, 2004; Vespa *et al.*, 2005; Dusick *et al.*, 2007; Hillered and Enblad, 2008). The reduction of CBF in TBI models is usually modest, not reaching ischemic levels (Muir *et al.*, 1992; Nilsson *et al.*, 1996), in agreement with the clinical observations.

Despite several shortcomings of rodent models, only a minority of all published TBI studies involves higher species and mice and rats will likely continue to be the dominant animal species in the future. In the next section, we discuss some additional factors influencing animal modelling in TBI.

Gender

In practically all cohort studies on TBI patients, a clear majority of TBI victims are males. In the clinical TBI literature, initial reports suggested that women did better following TBI than males although this has been questioned (Groswasser *et al.*, 1998; Farace and Alves, 2000; Bramlett and Dietrich, 2001; Ponsford *et al.*, 2008). Apparently, gender may play an important role because progesterone repeatedly improved outcome in experimental TBI studies and progesterone is currently undergoing clinical trial evaluation. Although most rodent studies have been performed in male rats, female animals are increasingly used to incorporate gender differences into experimental TBI models (Roof and Hall, 2000; Bramlett and Dietrich, 2001). Gender may also influence numerous aspects of the pathophysiology of TBI including the response to enriched environment following TBI in the rat and the degree of neurodegeneration (Kupina *et al.*, 2003; Wagner *et al.*, 2004; 2007; Bonatti *et al.*, 2008). Increased attention to gender aspects in TBI is clearly warranted.

Age

The mortality following TBI in the elderly patients is more than twice that of young patients (Mosenthal *et al.*, 2002) and age *per se* is one of the most important predictors of outcome after human TBI (Mosenthal *et al.*, 2002; Hukkelhoven *et al.*, 2003). In fact, the incidence of elderly patients with TBI appears to be increasing (Maas *et al.*, 2008). The vast majority of rodent TBI reports have been performed in adolescent or young adult animals with a rather narrow age range despite the fact that the past decade has seen a 21% increase in individuals over the age of 65 (Adekoya *et al.*, 2002). In the experimental setting, aged rats are more impaired in motor and cognitive performance compared to younger animals following lFPI and bifrontal contusions (Hamm *et al.*, 1992; Hoane *et al.*, 2004). Age-dependent injury-induced differences in gene expression in the hippocampus may contribute to the increased vulnerability of the aged rat brain to lFPI and mitochondria from aged animals are more perturbed compared with those of young animals (Shimamura *et al.*, 2004; Gilmer *et al.*, 2010). Pharmacological studies on aged animals are rare following TBI (Cutler *et al.*, 2007) and age-related preclinical TBI research still remains a severely underexplored area.

Species/strain differences and epigenetic changes

Strain differences in the response to TBI (lFPI) were recently reported between 3-month-old male Sprague–Dawley and Fisher rats, the latter showing a higher ICP, more seizure activity, longer ictal durations and more pronounced motor deficits, but surprisingly a better cognitive performance in a Morris Water Maze task (Reid *et al.*, 2010). Another study showed differences in cerebral immune cell infiltration and extent of brain damage following open skull WDI in two rat strains (Bellander *et al.*, 2010) and Sprague–Dawley rats had a more rapid behavioural recovery compared to Long–Evans rats following lFPI (Tan *et al.*, 2009; Reid *et al.*, 2010). Importantly, in a study on cyclosporine treatment following CCI, two different mice strains were evaluated for efficacy (Scheff and Sullivan, 1999). Accumulating evidence also suggest that various genetic factors are markedly important contributors to the pathophysiology and outcome of TBI patients (see Dardiotis *et al.*, 2010).

Epigenetic changes induced by TBI are also increasingly recognized (Gao *et al.*, 2006; Dash *et al.*, 2009) as a potentially important part of the secondary brain injury process. Thus, both epigenetic studies and the influence of strain on the outcome following experimental (and clinical) TBI is of very high interest in the TBI field and should be considered from a pharmacological view.

Neurocritical care and secondary insults

During the initial NCC period following human TBI, numerous secondary insults threaten the injured and vulnerable brain. These secondary injury factors can be divided into systemic (hypoxia, hypotension, anaemia, acid-base, electrolyte or glucose disturbances) or intracranial (raised ICP, seizures, impaired rCBF, hyperthermia) factors. These insults have only rarely been incorporated into existing TBI models. Additionally, good physiological monitoring (pO_2, pCO_2, pH, blood pressure, blood glucose, etc.) before and after TBI is often not used in the preclinical TBI models. Another commonly used clinical monitoring device is brain tissue oxygen monitoring, that is, Licox®, Paratrend® (Sarrafzadeh *et al.*, 2003) which has not, to date, been evaluated in experimental TBI. In contrast, *in vivo* cerebral microdialysis is used worldwide in the clinical setting and also in experimental TBI providing a possibility for translational research on, for example, energy metabolic perturbations following TBI (see Hillered *et al.*, 2005). A novel application of microdialysis is sampling of protein biomarkers directly in the injured brain following TBI using high molecular cut-off membrane

catheters and is receiving increasing attention in the NCC setting (Hillman *et al.*, 2005; Brody *et al.*, 2008; Helmy *et al.*, 2009; Marklund *et al.*, 2009; Dahlin *et al.*, 2010). Biomarker sampling directly in the injured brain and more closely to the pathoneurochemical process may improve the spatial and temporal resolution of the biomarker signals compared to traditional sampling in ventricular CSF or blood (Hillered *et al.*, 2005). Thus, microdialysis biomarker sampling may avoid many problems associated with long-distance transport, dilution and degradation causing a delay and reduction of the biomarker signals in CSF and blood. In support of this working hypothesis, our recent data in TBI patients indicate that the levels of the F_2-isoprostane 8-iso-$PGF_{2\alpha}$, a widely used biomarker of oxidative stress, were markedly higher in microdialysate compared with ventricular CSF and blood samples (F. Clausen *et al*, submitted).

Clinical NCC also uses numerous strategies to lower ICP, including administration of mannitol and hyperventilation, targeting physiological parameters. Sedatives and anaesthetics are commonly used in intensive care management of TBI patients. One elegant study using CCI in the rat showed that 'sub-optimal' cerebral perfusion pressure (CPP < 70 mmHg) resulted in an increased lesion volume (Kroppenstedt *et al.*, 1999) showing a good example of how scientists can take clinical observations for testing in the TBI laboratory. In the clinical setting, there is an ongoing search for prognostic and pathophysiological biomarkers (Dash *et al.*, 2010) that also should be evaluated in the experimental setting.

We emphasize that not only preclinical scientists are responsible for translating preclinical knowledge into clinical treatments. In numerous aspects of the management of TBI and, in particular, NCC there are discrepancies regarding, for example, sedation, fluid management and strategies to correct changes in ICP and CPP levels. These differences among neurosurgical and NCC centres indicate a lack of consensus and evidence-based guidelines making the animal modelling even more difficult. Clinicians obviously need to create international platforms working towards a more unified treatment strategy in the overall management of TBI. Encouragingly, international consortia and workshops have been created with the aim of creating, for example, management guidelines and uniform classification for TBI (e.g. Consensus conference, 1999; Saatman *et al.*, 2008; Compagnone *et al.*, 2005; Brain Trauma Foundation *et al.*, 2007; Margulies and Hicks, 2009), efforts that will likely be a key step in the improvement in TBI care worldwide.

Neurorehabilitation

Without doubt, neurorehabilitation is a very important part of the treatment of TBI victims. However, neurorehabilitation varies tremendously among countries and also regionally within many countries. Despite the differences in clinical rehabilitation, efforts for mimicking this aspect of the clinical management have been made. In the laboratory, numerous studies have evaluated enriched environment as a treatment option and consistently shown to enhance functional and histological recovery after both FPI and CCI in the adult rat (Hamm *et al.*, 1996; Passineau *et al.*, 2001; Hoffman *et al.*, 2008; Sozda *et al.*, 2010). Additionally, late effects of enriched environment plus multimodal early onset stimulation after TBI in rats resulted in an improved motor function without reduction of lesion volume (Lippert-Gruner *et al.*, 2007). The efficacy of drugs in TBI animals also subjected to enriched environment (Kline *et al.*, 2007) would be an interesting approach in order to further improve the clinical similarity of preclinical research.

Animal modelling in some previous and ongoing clinical trials

In the past, numerous extensive and expensive clinical trials have evaluated pharmacological compounds aiming to improve the recovery of TBI patients. Invariably, they all failed to demonstrate clinical efficacy. The reasons for these failures are multifactorial and have been addressed in the previous sections of this review (see also Maas *et al.*, 2008). Common shortcomings have included incomplete preclinical evaluation, incorrect timing and dosing of the selected compounds and the inclusion of too mildly or severely injured patients and inclusion of all the various subtypes of TBI. Still, numerous clinical trials in TBI are ongoing, examples being the ProTECT trial (progesterone; Wright *et al.*, 2007), aiming to enrol 1140 patients), the citicoline brain injury treatment trial (Zafonte *et al.*, 2009), aiming to enrol 1292 patients) and erythropoietin Phase II and III trials based on solid preclinical studies (NCT00375869 and NCT00313716; see Nichol *et al.*, 2009). Thus, it is important to learn lessons from past trials and also continuously evaluate trials that are ongoing or being initiated. Here we selected two examples, the magnesium and cyclosporine A trials with the aim of reviewing the published preclinical documentation. The reasons for selecting these two trials are that they both have unusually solid preclinical documentation prior to embarking on the clinical trials and the compounds are both in clinical use for other medical conditions. One of the trials is completed and the other is ongoing.

Magnesium

Magnesium (Mg^{2+}) is the second most abundant cation in the body, involved in more than 300 enzyme reactions (McKee *et al.*, 2005). Following experimental TBI, brain intracellular free Mg^{2+} concentration significantly declines in number of animal models evaluating mild-severe TBI in addition to focal and diffuse TBI and this decline was shown to correlate with the functional outcome (Heath and Vink, 1999a). In addition, in both humans and animals, TBI is associated with decreased serum Mg^{2+} levels. Mg^{2+} was suggested to enhance neuronal survival by, for example, blocking of the NMDA receptor ion channels. In a number of preclinical TBI studies, supplemental Mg^{2+} treatment consistently improved outcome in several different TBI models using a battery of functional outcome tests (Table 2). Because continuous Mg^{2+} infusion was safely used for other medical conditions including eclampsia and considered cheap and readily available, these encouraging animal data suggested that Mg^{2+} should be evaluated in a clinical setting. In a randomized, double-blind placebo-controlled monocentre clinical trial, 499 patients with moderate-severe TBI (GCS score of 3–12) were enrolled within 8 h post-injury (Temkin *et al.*, 2007). Mg^{2+} was administered by an initial i.v. dose followed by an i.v. infusion for 5

days and similar to the experimental findings, initial serum Mg^{2+} levels were low in 60% of patients. Due to initially high mortality and low blood pressure at the target serum Mg^{2+} level of 1.25–2.5 mmol·L^{-1} (normal levels are 0.75–1.0 mmol·L^{-1}), target serum levels were decreased to 1.0–1.85 mmol·L^{-1}. The included patients had various subtypes of TBI or a combination thereof; including DAI (36% of Mg^{2+}-treated patients), subdural haematoma (56%), EDH (21%), intracerebral haematoma (9%), depressed skull fracture (18%) and cortical contusions (60%) and a few patients had a penetrating TBI. Primary outcome was a composite of mortality, seizures, functional measures and neuropsychological tests. No improvement of Mg^{2+} treatment was observed, instead a clear negative trend in the outcome of Mg^{2+}-treated patients was observed. Numerous possible explanations for these disappointing and unexpected results were suggested, including a possible interaction with phenytoin and a possible negative effect of prolonged treatment (contrary to the preclinical setting) resulting in excessive NMDA receptor blockade. In our opinion, other possible explanations of the study results included the inclusion of all TBI subtypes, a too wide range of TBI severity and a prolonged time window without prior experimental supportive data. Importantly, rather high serum Mg^{2+} levels are well tolerated but produce a very modest increase in Mg^{2+} levels in the CSF (McKee *et al.*, 2005). The CSF Mg^{2+} was suggested to be increased in TBI *per se* (Kafadar *et al.*, 2007), suggesting a complex brain pharmacodynamic situation with regard to Mg^{2+} in humans. These aspects need to be considered in future TBI clinical trials.

Cyclosporin A

Cyclosporin A (CsA), known to inhibit T-cell lymphocytes by binding to cyclophilin A, has long been used in the clinical setting as an immunosuppressant to, for example, inhibit graft rejections following transplantation procedures. The CsA was suggested to influence TBI pathophysiology by binding to calcineurin, a known causative factor in the damage to the axonal cytoskeleton following TBI and positively influenced several aspects of cytoskeletal damage following TBI (Buki *et al.*, 1999; Okonkwo and Povlishock, 1999). The CsA was also suggested to inhibit the opening of the mitochondrial permeability transition pore although this mechanism of action has been questioned (Marmarou and Povlishock, 2006). The role of CsA as a neuroprotectant has been evaluated in several animal models of TBI (summarized in Table 2). The CsA does not reach the brain in high concentrations in non-TBI patients, since it is highly bound in the serum and is a substrate for multidrug resistance efflux pumps, eliminating CsA from the CNS compartment (Cook *et al.*, 2009). In TBI patients, CsA is detectable in the CSF for up to 6 days, suggesting that the increased permeability of the BBB after TBI may result in increased access for CsA to injured brain regions (Hatton *et al.*, 2008). Recently, the safety, tolerability and pharmacokinetics of CsA in TBI patients were evaluated. In 30 patients with severe TBI included within 8 h post-injury, CsA was injected twice daily using escalating doses up to 2.5 mg·kg^{-1} $dose^{-1}$ (Empey *et al.*, 2006). Compared with CsA pharmacokinetics in other disease states, a more rapid clearance and a larger distribution volume of CsA was demonstrated. Additionally, no significant differences between the placebo- and CsA-treated patients with regard to immunological parameters such as total lymphocyte count, the incidence of infections and T-cell subtypes were observed (Mazzeo *et al.*, 2006). In an additional study (Mazzeo *et al.*, 2009b), 50 patients with severe TBI received 5 mg·kg^{-1} infused over 24 h using continuous i.v. infusion initiated within 12 h of the injury. Overall, small and likely not clinically significant differences in renal function and white blood cell counts were observed in CsA-treated patients and the safety profile of CsA was good. However, no improvements in neurological outcome were observed at 3 or 6 months post-injury. Still, brain pharmacokinetics need to be carefully determined and it is yet unclear if all subtypes of TBI will be included in the next trial. A large Phase III efficacy trial of CsA in severe TBI is currently under peer review by the National Institute of Health-National Institute of Neurological Disorders and Stroke Clinical Trials study section, and if approved, will be performed in about 50 centres throughout the USA. The results of this study will be eagerly awaited by the TBI research community.

Conclusions

The lack of drugs with proven clinical efficacy in TBI is a major challenge ahead for the research community and the drug industry. So, where do we go from here? As outlined in this review, a successful translation of basic science knowledge to the clinic requires numerous refinements of the existing preclinical TBI models that may be achieved without extensive efforts or costs. We believe that the current experimental TBI models adequately reflect many aspects of human TBI. However, to more adequately mimic the clinical situation, modification of injury severity, refined functional outcome tests, addition of secondary insults and multimodality monitoring may be needed. In addition, more research into the effect of age, gender and species/strain on the outcome of TBI is warranted. On the clinical side the ongoing international effort to come up with a novel classification system for TBI patients is widely appreciated (see Saatman *et al.*, 2008) and may enable selection of more homogenous patient cohorts in future clinical trials. We also suggest that clinicians work toward an international consensus with a more homogenous treatment strategy for TBI patients in the NCC setting to facilitate multicentre comparisons. In addition, improved CNS drug delivery systems and monitoring of target drug levels and drug effects is warranted. Numerous promising treatment options have emerged in recent years, including neuroprotective, neurorestorative and anti-inflammatory compounds that should be subjected to a rigorous preclinical dose–response analysis of their efficacy on the target mechanism and the ability to reduce post-traumatic neurodegeneration and to improve behavioural and neurological recovery. Based on the complexity of injury mechanisms involved in TBI pharmacological combination treatment strategies may be an important option to consider.

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Conflict of interest

None.

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