Peptidergic Drugs for the Treatment of Traumatic Brain Injury

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Future Neurology. 2013;8(2):175-192.

Abstract and Introduction

Abstract

Traumatic brain injury (TBI) is a devastating medical condition that has an enormous socioeconomic impact because it affects more than 10 million people annually worldwide and is associated with high rates of hospitalization, mortality and disability. Although TBI survival has improved continuously for decades, particularly in developing countries, implementation of an effective drug therapy for TBI represents an unmet clinical need. All confirmatory trials conducted to date with drugs targeting a single TBI pathological pathway failed to show clinical efficacy, probably because TBI pathophysiology involves multiple cellular and molecular mechanisms of secondary brain damage. According to current scientific evidence of the participation of peptide-mediated mechanisms in the processes of brain injury and repair after TBI, peptidergic drugs represent a multimodal therapy alternative to improve acute outcome and long-term recovery in TBI patients. Preliminary randomized-controlled clinical trials and open-label studies conducted to date with the peptidergic drug Cerebrolysin® (Ever Neuro Pharma GmbH, Unterach, Austria) and with the endogenous neuropeptides progesterone and erythropoietin, showed positive clinical results. Cerebrolysin-treated patients had a faster clinical recovery, a shorter hospitalization time and a better long-term outcome. Treatment with progesterone showed advantages over placebo regarding TBI mortality and clinical outcome, whereas erythropoietin only reduced mortality. Further validation of these promising findings in confirmatory randomized-controlled clinical trials is warranted. This article reviews the scientific basis and clinical evidence on the development of multimodal peptidergic drugs as a therapeutic option for the effective treatment of TBI patients.

Introduction

Traumatic brain injury (TBI) affects 10 million individuals annually worldwide and represents a major health and socioeconomic problem. The highest incidence of TBI is among young (15–25 years old) and old (75 years and older) individuals, and it is the first cause of injury-related death and disability in children and young adults; it accounts for the hospitalization of approximately 100 cases per 100,000 population/year and for an annual death rate of 18 deaths/100,000 population, which is even greater in low-income countries. Furthermore, it enhances the risk of death for at least 7 years after hospitalization and causes long-term disabilities in more than 1% of the population.[1–4]

Although the survival rate of TBI cases has improved continuously for decades, particularly in developing countries, there has been no real progress in the prevention and management of the lifelong impairments induced by TBI on physical, cognitive and psychosocial functioning.[3] Apart from reducing the high mortality in severe cases and in elderly patients, the main goal of TBI treatment is the secondary prevention of long-lasting disabilities by enhancing brain recovery after TBI. Recent advances in the characterization of the cellular and molecular mechanisms involved in TBI pathophysiology has allowed novel therapeutic targets to be identified, but almost all drug trials conducted to date have failed to demonstrate clinical efficacy.[5] Therefore, at present, there is no specific drug therapy approved for TBI.

In this article, the authors reviewed recent pharmacological and clinical studies supporting the development of peptidegic drugs as a promising option for TBI treatment.

Traumatic Brain Damage: Multiple Pathologic Processes

Traumatic brain damage involves multiple pathologic processes underlying the primary and secondary mechanisms of injury. The external impact on the head produces immediate mechanical insults that may result in direct neurovascular damage and disruption of the blood–brain barrier (BBB). Primary lesions trigger a complex cascade of reactive events leading to acute and/or persistent mechanisms of delayed brain injury mediated by cerebral edema,[6,7] ischemia–hypoxia,[8–10] metabolic dysfunction,[11–13] excitotoxicity,[10,12,14] oxidative stress,[10,15,16] neuroinflammation,[14,15,17–19] apoptosis and delayed neural damage,[15,20–22] alterations in hormones,[23,24] neurotrophic factors[14,25–27] and proteinopathies.[18,28–30] These pathologic processes are amenable to therapeutic interventions for brain protection and repair intended to prevent secondary lesions.

Some of the key molecules involved in the secondary mechanisms of traumatic brain damage and constituting potential drug targets are: glutamate and its receptors,[31–33] calpain,[34] caspases,[35] proinflammatory cytokines such as TNF-α, IL-1β and IL-6,[6,36,37] reactive oxygen species,[38,39] β-amyloid (Aβ) and tau-proteins,[40–42] neurotrophic factors such as brain-derived neurotrophic factor (BDNF), IGF-1 or VEGF,[43–46] and signaling proteins including kinases such as Akt and GSK-3β.[47,48]

The interaction of primary and secondary brain injury mechanisms may give rise to microhemorrhages, hematomas, vasospasm, vascular necrosis, vasogenic edema, cytotoxic swelling, BBB leakage, neuronal loss, cytoskeletal and axonal damage, decreased neural connectivity, gliosis, chronic activation of reactive microglia, abnormal accumulation of neurodegeneration-related proteins (neurofilament proteins, tau, synuclein, ubiquitin, progranulin, TDP43, amyloid precursor protein and Aβ) and reduced neuroplasticity (angiogenesis, neurogenesis, synaptogenesis and axon–dendritic remodeling). Structural and functional studies on biomarkers of these alterations provide insight into the pathophysiology of TBI and supportive information for the diagnosis, prognosis and treatment monitoring of TBI patients.[10,13,19–21,25–30,43,49–64]

Peptidergic Mechanisms, Neurodegeneration & Neurorepair After TBI

Neuroinflammation: Microglia Activation & Cytokines

Neuroinflammation is one of the main components of TBI pathophysiology, contributing to both secondary damage and recovery mechanisms. TBI elicits an immediate inflammatory response characterized by an increased production of proinflammatory cytokines and activation of microglial cells. Recent studies demonstrated a temporal pattern of upregulation of proinflammatory cytokines (i.e., TNF-α, IL-1β, IL-6 and IL-8) in the brain extracellular fluid 1–2 days after TBI, with later elevations or no changes in the levels of the anti-inflammatory cytokine IL-10.[17,65] Similar elevations of proinflammatory cytokines without upregulation of anti-inflammatory cytokines were also found in post-mortem brain tissue, cerebrospinal fluid (CSF) and blood of TBI patients.[66,67] Activated microglia have been observed in human TBI brains from 3 days after injury[18] and, as assessed by PET, showed a widespread increase in the brain consistent with diffuse neuronal damage 6 months after TBI,[53] and were enhanced in subcortical regions, but not at the original site of focal brain lesion, up to 17 years after TBI.[19]

Acute microglia activation might contribute to neuroprotection after injury through the release of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist, and of growth factors such as NGF.[18,37] A subpopulation of activated microglia that are immunoreactive for galectin-3/Mac-2 and NGF was recently reported to be upregulated shortly after diffuse axonal injury in mice.[68] In humans, higher levels of NGF in the CSF and increased IL-1 receptor antagonist concentrations in brain microdialysates were found to be associated with improved TBI outcomes.[51,69,70] On the other hand, the chronic production of proinflammatory cytokines, nitric oxide and superoxide species by activated microglia seems to contribute to the maintenance of the secondary brain damage mechanism induced by TBI.[18,37] In fact, lower CSF levels of IL-1β were associated with a better outcome in TBI children,[69] whereas increased serum IL-6 levels were associated with unfavorable outcomes[71] and a higher risk of developing elevated intracranial pressure.[54] High circulating levels of soluble TNF receptors showed a predictive capacity for the development of late-onset multiple organ failure after traumatic injury.[72] Inflammatory mediators have been

involved in the induction of cerebral edema, vascular permeability, BBB disruption and excitotoxic and apoptotic cell death after brain injury.[6,31,35,73,74] Recently, it was shown that neutralization of IL-1β attenuates TBI-induced edema, tissue loss and learning impairment in mice.[36] TNF may produce excitotoxic and apoptotic damage after TBI by altering the expression of AMPA ionotropic glutamate receptors[31] and by enhancing caspase-dependent apoptosis through TNF receptor-1)/Fas signaling.[75] However, the combination of TNF receptor-2/Fas receptors showed a beneficial role for the recovery of motor and cognitive function after TBI.[75]

Hormones: Progesterone, Growth Hormone & IGF-1

Endocrine dysfunctions are very common in TBI patients during the first 7–10 days postinjury,[24,76] and increased estradiol and testosterone levels over this time period were found to be associated with increased mortality and a worse outcome for both men and women with severe TBI.[24] Deficits of single or multiple pituitary hormones were detected in more than 20% of the TBI patients 1 year after injury, with hypogonadism, growth hormone (GH) deficiency and low circulating levels of IGF-1 being the most frequent findings.[23,77] Enduring low levels of gonadal steroids, GH and IGF-1 may have a negative impact on the mechanisms of neurorepair following TBI, given the putative neuroprotective functions of these hormones.[44,78–80] In fact, post-traumatic hypopituitarism was found to be associated with an unfavorable lipid profile and a worse quality of life at 1 year after TBI.[81] However, cognitive impairment was not found to be associated with GH and IGF-1 deficits in adult patients tested more than 1 year post-TBI.[82]

Recent experimental studies indicate that the neurosteroid progesterone may exert neuroprotective effects in TBI by reducing apoptotic proneurotrophin signaling, cellular apoptosis, inflammation and cerebral edema, and by enhancing prosurvival neurotrophin signaling, vascular remodeling and BBB protection.[79,80,83–85] Treatment with progesterone after TBI reduced the brain levels of the proapoptotic precursor proteins of NGF (pro-NGF) and BDNF (pro-BDNF), increased the levels of the anti-apoptotic mature NGF, reduced the expression of apoptosis markers and improved behavioral parameters in rats with bilateral frontal cortical contusions.[83] However, in the same study, progesterone also reduced the expression of mature BDNF and its TrkB receptor, which are essential for neuroplasticity during brain repair.[83] In rats with unilateral parietal cortical contusion, progesterone increased the circulating levels of endothelial progenitor cells, facilitated vascular remodeling and improved neurological outcome.[84] In another animal model of TBI, progesterone influenced the brain levels of inflammatory cytokines (i.e., IL-1β, IL-6, TNF-α and TGF-β) in a differential dose- and time-related manner, thus reducing IL-6 and TNF-α, but enhancing TGF-β and IL-1β after injury.[85]

Based on experimental data, it has been suggested that women compared with men, and pregnant versus nonpregnant women, may have more favorable outcomes after TBI owing to the neuroprotective effects of higher estrogen and progesterone levels; however, recent clinical studies did not confirm this assumption.[86–88] Pregnant patients with moderate-to-severe TBI showed no differences in mortality compared with their nonpregnant counterparts, but rather a trend toward increased mortality.[86] In a retrospective study comparing hormonally active women with men, no differences in sex-related mortality were found, and brain edema tended to be more frequent in females.[88] An even lower rate of survival was observed in females compared with males with severe TBI.[87]

GH and IGF-1 also have neuroprotective actions, promoting neuroplasticity and influencing the genesis and regeneration of cells in the adult brain.[44,78] IGF-1 acts on its receptor activating PI3K/Akt or pMAPK/ERK intracellular signaling pathways. Upregulated levels of IGF-1, the receptor for IGF-1 and its downstream signaling mediators were found in the hippocampus, cortex, subcortical white matter and cortical vessels in different rodent TBI models.[89–92] This IGF-1 upregulation seems to reflect an endogenous neuroprotection/neurorepair response as indicated by its protective affect on the survival of hippocampal CA1 neurons through Akt activation.[92] On the contrary, decreased serum levels of GH and IGF-1 were found after TBI in adult and inmature rats, respectively.[93,94] Low circulating IGF-1 levels were associated

with hippocampal neuron loss and spatial memory deficits,[94] whereas peripheral GH depletion was associated with persistent inflammatory changes in the brain.[93] These data, together with findings that the peripheral administration of IGF-1 analogs improves somatosensory–motor function and long-term histological outcome after brain injury,[44] support the contribution of GH and IGF-1 to the TBI mechanisms of degeneration and repair.

Trophic Factors: BDNF, NGF & VEGF

BDNF and NGF are neurotrophins that promote neuronal survival and plasticity through binding to their specific high-affinity tyrosine kinase receptors (TrkB and TrkA, respectively) and the common low-affinity p75 neurotrophin receptor as well as the activation of downstream PI3K/Akt and MAPK/ERK signaling pathways. However, their precursor proteins (pro-BDNF and pro-NGF) induce apoptosis by coupling to the sortilin and the p75 neurotrophin receptor death-signaling receptor complex.[45]

Human TBI studies found that serum BDNF and NGF levels decreased in young adults as the severity of the injury increased,[27] and that elevated CSF levels of NGF, but not BDNF, correlated with TBI severity and were associated with a better outcome in children.[51] Recent genetic investigations demonstrated associations of some *BDNF* gene polymorphisms with clinical aspects of TBI outcome. The Val66Met *BDNF* polymorphism influenced the recovery of executive functioning after penetrating TBI[95] and the response to treatment with citalopram in depression secondary to TBI,[96] but not the cognitive performance 1 month after mild TBI.[97] Two single-nucleotide *BDNF* polymorphisms were significantly associated with postinjury recovery of general cognitive intelligence,[98] and another four were associated with memory measures 1 month after mild TBI.[97]

The reductions of BNDF, TrkB receptor and the downstream effectors on synaptic plasticity, learning and memory (synapsin 1, CREB and α-CAMKII) observed in injured animal brains suggest an inhibition of the endogenous trophic activity after TBI, particularly in the peritraumatic area.[39,99–101] Interestingly enough, increased BDNF and synapsin 1 within the cortex contralateral to the lesion may reflect compensatory restorative processes in areas homotypical to the injury,[99] which is in agreement with the recent demonstration that endogenous BDNF is essential for the recovery of motor function following unilateral brain injury by inducing reorganization of the corticospinal tract through contralateral sprouting fibers.[102] The potential contribution of BDNF to processes of neurorepair after TBI is highlighted by experimental studies demonstrating that its administration upregulates neuroprotective genes in CA3 hippocampal neurons similar to those genes induced by injury,[103] whereas the blockade of its TrkB receptors blunts the increases of BDNF, synapsin 1 and CREB induced by exercise in rats.[104] Further support for the protective effects of BDNF in TBI arises from pharmacological studies demonstrating that: BDNF mimetics activate TrkB receptors and improve learning after TBI in rats;[46] human mesenchymal stem cells seem to improve TBI functional recovery by increasing the secretion of BDNF and other neurotrophic factors and reducing apoptosis;[105] and that *S*-nitrosoglutathione protects axonal integrity and promotes synaptic plasticity at the same time that it enhances BDNF and TrkB expression in the TBI brain.[39]

VEGF is upregulated after TBI and participates in several processes of brain repair:[25,43,57,106–110] it has trophic and protective effects on neurons; it stimulates astroglial mitosis, scar formation and production of growth factors; it promotes endothelial cell survival, angiogenesis, vascular remodeling and BBB repair helping to re-establish metabolic and trophic support to the injured tissue.

Elevated levels of VEGF in the brain and serum were found in severe TBI patients during the first 1–3 weeks postinjury.[25,43,109] The peripheral upregulation of VEGF was associated with an increase of circulating endothelial progenitor cells, which suggests its involvement in angiogenesis and vascular repair after TBI.[25,43] In TBI animal models, serum and brain VEGF levels were also found to be upregulated.[106,108]

VEGF acts through its two tyrosine kinase receptors; flt-1 (VEGF-R1) expressed by vascular endothelial cells and activated astrocytes, and flk-1 (VEGF-R2) expressed on vascular endothelium and some

neurons.[107] After penetrating TBI, VEGF-R1 was upregulated in reactive astrocytes and its neutralization reduced astroglial mitogenicity, scar formation and expression of growth factors (ciliary neurotrophic factor and FGF), and caused some increase in endothelial degeneration; conversely, VEGF-R2 was upregulated in neurons and its blockade reduced vascular proliferation around the wound and increased endothelial cell degeneration, without effects on astrogliosis and growth factor expression.[107] These data suggest that upregulation of VEGF after TBI may induce angiogenesis and astroglial proliferation, expression of growth factors and scar formation through activation of VEGF-R1. Finally, the involvement of VEGF in brain protection and repair after TBI is also supported by the recent finding that its exogenous (intracerebroventricular) administration resulted in increased neurogenesis and angiogenesis, reduced lesion volume and improved functional outcome in TBI mice.[110]

Alzheimer's Disease-related Proteins: Aβ & Tau

TBI and Alzheimer's disease (AD) share several pathologic mechanisms including the abnormal accumulation of Aβ and tau proteins. Severe TBI is considered a major risk factor for the later development of AD.

Brain Aβ is elevated acutely after TBI and its intra-axonal and extracellular (Aβ plaques) accumulation may contribute to secondary injury mechanisms including neuronal apoptosis, axonal damage and microglia activation, among others. Increased levels of tau and the axonal and intracytoplasmic accumulation of its aggregates are also involved in TBI pathophysiology.

Autopsy studies found Aβ plaques in approximately 30% of TBI victims[111] and these deposits developed rapidly after injury,[111,112] were associated with higher levels of soluble $A\beta_{42}$ [113] and were influenced by a genetic polymorphism of neprilysin, a major Aβ-degrading enzyme.[114] Acutely after TBI, Aβ also showed a remarkable intra-axonal co-accumulation with amyloid precursor protein and enzymes necessary for Aβ genesis, including BACE1 protein (β-secretase) and the γ-secretase complex protein, presenilin-1.[112,115] $A\beta_{42}$ levels were also found to be increased in ventricular CSF,[116] but decreased in lumbar CSF after TBI.[117,118] Interestingly, a rise of interstitial Aβ after acute TBI was found to correlate positively with improvements in the neurological status, as assessed by the Glasgow Coma Score (GCS), and with brain glucose levels, and negatively with elevated intracranial pressure and with tissue hypoxia markers.[28] These findings suggest that a reduced release of soluble Aβ may reflect an increased accumulation of Aβ in patients with more severe brain damage and a worse neurological functioning. In fact, reduced levels of interstitial Aβ were associated with low EEG activity after experimental TBI,[119] and with a progressive deposition of Aβ in the brain parenchyma during aging.[120] Experimental pharmacological studies demonstrated that reductions in Aβ accumulation were associated with improved brain pathology, decreased microglia activation and better outcome after TBI.[41,121,122]

Tau-related pathology is present in the brains of patients with chronic traumatic encephalopathy or acute TBI,[55,115] and increased levels of the tau protein were found in the brain, CSF and serum after TBI in humans.[29,123–125] The levels of tau in the brain extracellular fluid rose shortly after TBI, and this tau increase was associated with low Aβ levels and worse clinical outcome in patients with severe TBI.[29] Initial elevations of CSF and serum tau levels were also found to be predictors of poor clinical outcome in severe TBI.[123–125]

Other Peptide-related Mechanisms: The PI3K/Akt/GSK-3β Signaling Pathway

TBI produces neuronal damage and deficits in neuroplasticity, and the capacity of injured neurons to regenerate is modulated to some extent by changes in the expression of intracellular signaling molecules such as Akt and GSK-3β.[47,48,126] GSK-3β is a constitutively active kinase involved in neuronal apoptosis, tau hyperphosphorylation and other pathologic processes relevant for TBI. Neurotrophic factors are major regulators of GSK-3β activity. Growth factors act on their receptors, inducing PI3K to phosphorylate Akt by activating it, which in turn leads to GSK-3β inactivation through direct phosphorylation. The PI3K/Akt/GSK-

3β intracellular signaling pathway plays a central role in the regulation of different neuronal functions by neurotrophic factors, and there is evidence of its involvement in the reduction of Aβ production and tau hyperphosphorylation,[127] the modulation of neuroinflammation,[128] the increase of brain IGF-1 levels,[129] the uptake of cellular glucose,[130] the protection against neuronal damage, apoptosis and death,[131–134] the promotion of neuroplasticity and neurogenesis[133,134] and the improvement of cognitive deficits in different experimental conditions.[129] GSK-3β inhibition and Akt activation were also found to contribute to the enhancement neuronal survival, learning and memory after TBI.[48]

Neuropeptides: A Multifunctional Treatment Option in Neurotrauma

Most of the therapies investigated to date in TBI have failed, probably because they used drugs targeted toward a single pathological factor and the pathogenesis of TBI involves multiple cellular and molecular mechanisms. Many researchers now propose that multifunctional drugs constitute the most appropriate alternative for TBI treatment.[35,80,135,136] Neurotrophic factors are pleiotropic agents with pluripotential activities on multiple molecular pathways and cellular processes that are relevant for TBI pathology and recovery (Figure 1).[125,137–139] Neurotrophins and other trophic factors have well-established actions in regulating apoptosis and cell survival, angiogenesis, neurogenesis and neuroplasticity (cytoskeleton restructuring, dendritic sprouting and remodeling, and synaptogenesis),[131–134,137–139] and are essential for restoring the integrity of the neurovascular unit and for the peptide modulation of periventricular neurogenic regions after TBI.[26,140]

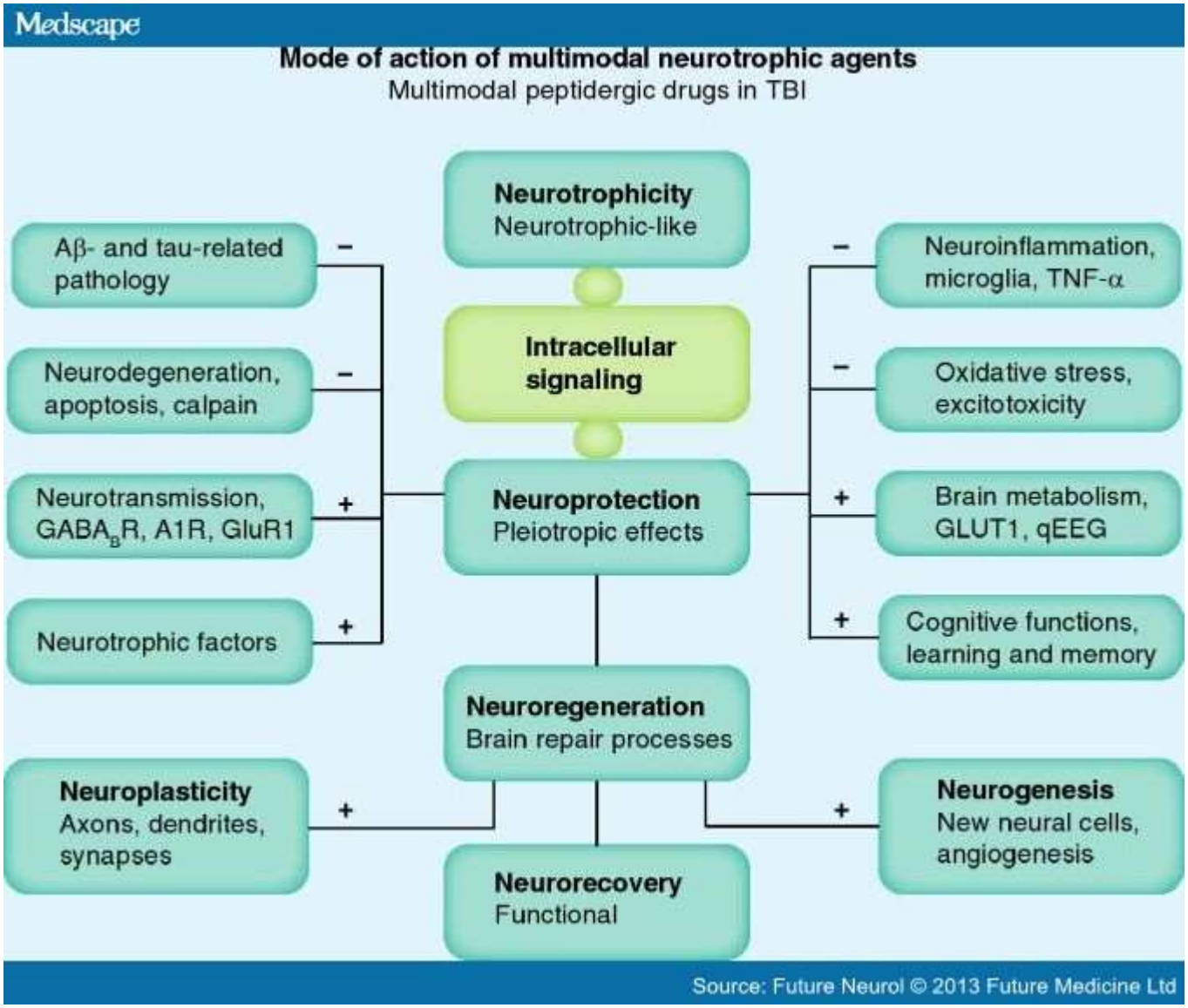

Figure 1.

Pleiotropic effects on multiple traumatic brain injury pathophysiological mechanisms and multimodal activity on neuroprotection, brain repair and recovery processes.

+: Increase or improvement; -: Decrease or inhibition; A1R: Adenosine A1 receptor; Aβ: β-amyloid; $GABA_BR$: GABA B receptor; GluR1: Glutamate receptor subunit 1; qEEG: Quantitative EEG; TBI: Traumatic brain injury.

Adapted with several modifications from [144].

Although trophic factors are good candidates for TBI treatment, their therapeutic use has several important limitations, such as their rapid enzymatic inactivation and low uptake through the BBB. Therefore, pleiotropic and multimodal peptidergic drugs need to be developed that act on multiple TBI pathophysiological pathways to induce neuroprotective effects, and that have the capacity to stimulate brain repair and regeneration after injury (Figure 1). Several endogenous peptides (i.e., progesterone and

erythropoietin [EPO]), naturally cleaved peptides (Cerebrolysin® [Ever Neuro Pharma GmbH, Unterach, Austria] and BDNF- and IGF-1-derived peptides), synthetic analogs and mimetic peptides are being investigated as promising TBI therapies.[41,43,44,46,121,141] Antagonists of kinins (bradykinin) and tachykinins (substance P), and particularly substance P neurokinin-1 receptor antagonists, represent another category of TBI therapeutic agents under active investigation, a discussion of which exceeds the scope of the present review.[5,6,136]

Treatment of TBI With Peptidergic Drugs: Clinical Studies

The activation of the endogenous mechanisms of neuroprotection and neuroplasticity is not sufficient to counteract the secondary brain damage and the enduring functional deficits produced by TBI. Thus, implementation of an effective therapy for TBI represents an unmet clinical need. Multimodal peptidergic drugs intended at promoting brain repair and regeneration processes seem to constitute a good therapeutic option to improve acute outcome and long-term recovery of TBI patients.

Two important aspects to be considered regarding TBI treatment are the acute management of elderly patients and the therapeutic time window for long-term recovery. The poor prognosis of older patients after TBI suggests an altered pathophysiological response in the aging brain,[1,2] which is supported by the recent findings of more intense and prolonged alterations of brain metabolism (reduced glucose levels), neuronal ischemia (enhanced lactate/pyruvate ratio) and cell damage (increased levels of glycerol and glutamate in elderly patients compared with young patients with TBI).[14,142] A reduction in the cerebral VEGF response elicited by TBI was also found in elderly rats.[106] These age-associated differences may influence the therapeutic response of elderly TBI patients. On the other hand, the persistence of brain alterations such as microglia activation,[19,53] intra-axonal amyloid deposition[112] and glucose hypometabolism[143] during months to years postinjury indicate that some secondary injury mechanisms are maintained and could be amenable to multimodal treatment. Finally, the recent observation of a chronic release of MAP-2 into the circulation of TBI patients with higher levels of consciousness is indicative of sustained neuroplasticity after TBI,[56] and further supports the long-term use of multimodal therapies.

Cerebrolysin: A Multimodal Peptidergic Drug

Cerebrolysin is a peptidergic drug with neuroprotective and neurorestorative properties, consisting of low molecular weight peptides that are able to cross the BBB and mimic the action of endogenous neurotrophic factors.[144–146] Some of the neuropeptides contained in the Cerebrolysin concentrate demonsrated cross-reactivity with antibodies against several trophic factors (glial cell line-derived neurotrophic factor, ciliary neurotrophic factor, IGF-1 and IGF-2), suggesting their potential capacity to bind receptors of these factors. Cerebrolysin acts as a multimodal drug exerting, probably through synergistic actions of its peptides, pleiotropic positive effects on Aβ and tau pathologies, neuroinflammation, neurotrophic factors, oxidative stress, excitotoxicity, neurotransmission, brain metabolism, neuronal apoptosis and degeneration, neuroplasticity, neurogenesis as well as cognition, as shown in experimental and human studies.[144–146]

In pharmacodynamic studies it has been demonstrated that Cerebrolysin:

- Reduces brain Aβ deposition, tau phosphorylation and Aβ- and tau-related neuropathology by regulating GSK-3β and CDK-5 activity;[147–149]
- Modulates neuroinflammation, attenuating microglia activation and IL-1β release *in vitro* and *in vivo*, and reducing the elevated serum levels of TNF-α and TNF receptor-1 in AD patients;[150–153]
- Displays neurotrophic-like actions on neuronal survival and neurite outgrowth and increases circulating IGF-1 and BDNF levels in humans;[144,151,154–157]

- Protects against oxidative and excitotoxic damage, at least in part by inhibiting lipid peroxidation and calpain activation;[158–162]
- Influences synaptic transmission mediated by $GABA_B$, adenosine A1 and glutamate receptor subunit 1 receptors and exhibits cholinotrophic activity;[154,157,163–166]
- Enhances the supply of glucose to the brain and ameliorates the slowing of brain bioelectrical activity;[167–170]
- Promotes neural plasticity and prevents dendritic and synaptic loss;[155,157,171–178]
- Promotes neuronal survival protecting neurons from apoptosis and degeneration;[147–149,154–157,159,176–179]
- Stimulates neurogenesis, probably through Akt activation;[180–184]
- Improves learning and memory.[147,148,164,170,177,182,185–187]

The pleiotropic effects of Cerebrolysin are consistent with a putative neurotrophic-like mode of action of the drug exerted through, at least, activation of the PI3K/Akt/GSK-3β intracellular signaling pathway. The pharmacological activities of Cerebrolysin include not only neurotrophicity and several mechanisms of neuroprotection, but also neuroplasticity and neurogenesis, which are essential for the repair and regeneration of brain damage (Figure 1). In fact, Cerebrolysin reduced BBB and blood–CSF barrier leakage, attenuated brain edema and neuronal damage and improved sensory–motor functions in a rat model of TBI.[141] These findings are in agreement with the protective effects displayed by Cerebrolysin in other animal models of brain injury induced by intrahippocampal implants of Aβ,[147] fimbria–fornix transection,[154,185] septohippocampal pathway disruption,[159] neonatal ventral hippocampal lesion[177] or bilateral cortical lesions.[187] Recent randomized, double-blind, placebo-controlled clinical trials also demonstrated the clinical efficacy of Cerebrolysin in AD and vascular dementia patients,[188–190] as well as a significant reduction in the death rate in severe stroke patients treated with Cerebrolysin.[191] Therefore, Cerebrolysin is a good drug candidate for the effective treatment of TBI patients.

Effects of Cerebrolysin on Clinical & Biological Parameters in TBI Patients

The effects of Cerebrolysin on clinical and biological parameters during acute and postacute TBI phases were evaluated in several trials. Data of randomized controlled trials (RCTs) on the effects of Cerebrolysin in acute TBI are summarized in .

Table. Clinical studies with the peptidergic drug Cerebrolysin® in acute traumatic brain injury treatment.

Study design (year)	Study characteristics	Treatment regimen	Main outcome(s)	Ref.
Prospective, double-blind, placebo-controlled trial				
König *et al.* (2006)	Acute moderate–severe TBI (GCS: 4–11) Cerebrolysin® (Ever Neuro Pharma GmbH, Unterach, Austria; n = 22), placebo (n = 22) End point: 3 weeks Follow-up: weeks 6	Daily iv. infusions of Cerebrolysin 50 ml or placebo for 21 days in patients hospitalized within 6 h after TBI	End point: no GCS and CGI differences; faster GCS (p = 0.007) and better SKT (p = 0.030) improvements with Cerebrolysin Follow-up: superiority of Cerebrolysin in SKT performance (week 6: p = 0.045; week 9: p = 0.024)	[192]

	and 9			
Prospective, open-label trial: randomized vs placebo				
Wang *et al.* (1998)	Acute TBI (average GCS: 9) Cerebrolysin (n = 111; 68 surgery cases), placebo (n = 89; 52 surgery cases) End point: day 10	Daily iv. infusions of Cerebrolysin 20 ml (10 ml in children) vs vehicle (5% glucose solution, 250 ml) for 10 days Start within 24 h after TBI	More patients ($p < 0.05$) on Cerebrolysin improved in the global level of consciousness (surgery: 65 vs 38%; no surgery: 51 vs 32%) and the GCS (surgery: 91 vs 62%; no surgery: 91 vs 59%)	[193]
Prospective, open-label trials: randomized vs basic standard therapy				
Duma and Mutz (1990)	Severe–acute TBI (GCS: <9) Cerebrolysin plus dextran-40 (Ceredex; n = 20), standard therapy (n = 20) End point: at hospital discharge	Four iv. infusions/day of Cerebrolysin 10 ml plus dextran-40 250 ml add-on to standard therapy vs standard therapy alone until discharge	Ceredex induced earlier hospital discharge ($p < 0.01$), and significant improvements in cerebral perfusion pressure ($p < 0.01$) and rapid fatigue ($p < 0.05$) Three cases died in each group	[196]
Fei and Shenyui (1992)	Severe–acute TBI (GCS: 5–8) Cerebrolysin (n = 20), basic therapy (n = 20) End point: day 7 Follow-up: 6–9 months post-TBI	Daily iv. infusions of Cerebrolysin 10 ml add-on to basic therapy vs basic therapy alone for 7 days Start within 24 h after TBI	End point: significant treatment differences for the reduction of MDA levels ($p < 0.05$) and the GCS improvement ($p < 0.05$) Follow-up: better outcome in Cerebrolysin-treated patients (GOS: $p < 0.05$)	[194]
Zhou and Yang (1993)	Mild-to-severe–acute TBI Cerebrolysin (n = 30), basic therapy (n = 30) End point: day 15	Daily iv. infusions of Cerebrolysin 20–30 ml add-on to basic therapy vs basic therapy for 10–15 days Start within 24 h after TBI	Significant improvements ($p < 0.05$) with Cerebrolysin in GCS and in auditory evoked potentials at end point	[195]

CGI: Clinical Global Impression; GCS: Glasgow Coma Score; GOS: Glasgow Outcome Score; iv.: Intravenous; MDA: Malondialdehyde (metabolite of lipid peroxidation and oxidative stress marker); SKT: Syndrom-Kurz test; TBI: Traumatic brain injury.

In the only RCT conducted so far with Cerebrolysin add-on therapy in acute TBI patients ( ), König *et al.* found no significant group differences in the GCS and the Clinical Global Impression score at end point (day 21) because all patients improved, irrespectively of the treatment with Cerebrolysin or placebo.[192] However, compared with patients on placebo, Cerebrolysin-treated patients had a faster clinical recovery before end point, as indicated by treatment differences in the improvements over time of consciousness/vigilance ($p < 0.001$), global GCS severity ($p < 0.01$), GCS items such as 'eye opening' ($p < 0.05$), 'best verbal response' ($p < 0.05$) and 'best motor response' ($p < 0.01$). Furthermore, Cerebrolysin-treated patients showed a marginally significant ($p < 0.059$) earlier and higher Clinical Global Impression improvement over the whole 9-week study period and achieved significantly better ($p < 0.05$) cognitive improvements in the Syndrom-Kurz test at end point, and at follow-up at weeks 6 and 9 ( ). In an open-label, placebo-controlled study ( ), Wang *et al.* also found a faster recovery of consciousness and GCS scores in acute TBI patients after 10 days of Cerebrolysin treatment ( ).[193] Similar GCS improvements were

reported at 7 and 15 days postinjury in patients treated with Cerebrolysin.[194,195] All these findings are in agreement with the earlier hospital discharge induced by Cerebrolysin compared with standard therapy (9.9 vs 12.7 days; $p < 0.01$) in severe TBI patients.[196] A positive effect of acute Cerebrolysin therapy on long-term outcome, as assessed by the Glasgow Outcome Score (GOS), was observed 6–9 months after severe TBI in the only RCT evaluating it.[194] However, other authors found no significant ($p = 0.065$) differences in 6-month extended GOS compared with a historical cohort.[197]

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Prospective, open-label trial: randomized vs placebo				
Wang *et al.* (1998)	Acute TBI (average GCS: 9) Cerebrolysin (n = 111; 68 surgery cases), placebo (n = 89; 52 surgery cases) End point: day 10	Daily iv. infusions of Cerebrolysin 20 ml (10 ml in children) vs vehicle (5% glucose solution, 250 ml) for 10 days Start within 24 h after TBI	More patients ($p < 0.05$) on Cerebrolysin improved in the global level of consciousness (surgery: 65 vs 38%; no surgery: 51 vs 32%) and the GCS (surgery: 91 vs 62%; no surgery: 91 vs 59%)	[193]
Prospective, open-label trials: randomized vs basic standard therapy				
Duma and Mutz (1990)	Severe–acute TBI (GCS: <9) Cerebrolysin plus dextran-40 (Ceredex; n = 20), standard therapy (n = 20) End point: at hospital discharge	Four iv. infusions/day of Cerebrolysin 10 ml plus dextran-40 250 ml add-on to standard therapy vs standard therapy alone until discharge	Ceredex induced earlier hospital discharge ($p < 0.01$), and significant improvements in cerebral perfusion pressure ($p < 0.01$) and rapid fatigue ($p < 0.05$) Three cases died in each group	[196]
Fei and Shenyui (1992)	Severe–acute TBI (GCS: 5–8) Cerebrolysin (n = 20), basic therapy (n = 20) End point: day 7 Follow-up: 6–9 months post-TBI	Daily iv. infusions of Cerebrolysin 10 ml add-on to basic therapy vs basic therapy alone for 7 days Start within 24 h after TBI	End point: significant treatment differences for the reduction of MDA levels ($p < 0.05$) and the GCS improvement ($p < 0.05$) Follow-up: better outcome in Cerebrolysin-treated patients (GOS: $p < 0.05$)	[194]

Zhou and Yang (1993)	Mild-to-severe–acute TBI Cerebrolysin (n = 30), basic therapy (n = 30) End point: day 15	Daily iv. infusions of Cerebrolysin 20–30 ml add-on to basic therapy vs basic therapy for 10–15 days Start within 24 h after TBI	Significant improvements ($p < 0.05$) with Cerebrolysin in GCS and in auditory evoked potentials at end point	[195]

CGI: Clinical Global Impression; GCS: Glasgow Coma Score; GOS: Glasgow Outcome Score; iv.: Intravenous; MDA: Malondialdehyde (metabolite of lipid peroxidation and oxidative stress marker); SKT: Syndrom-Kurz test; TBI: Traumatic brain injury.

Table. Clinical studies with the peptidergic drug Cerebrolysin® in acute traumatic brain injury treatment.

Study design (year)	Study characteristics	Treatment regimen	Main outcome(s)	Ref.
Prospective, double-blind, placebo-controlled trial				
König *et al.* (2006)	Acute moderate–severe TBI (GCS: 4–11) Cerebrolysin® (Ever Neuro Pharma GmbH, Unterach, Austria; n = 22), placebo (n = 22) End point: 3 weeks Follow-up: weeks 6 and 9	Daily iv. infusions of Cerebrolysin 50 ml or placebo for 21 days in patients hospitalized within 6 h after TBI	End point: no GCS and CGI differences; faster GCS ($p = 0.007$) and better SKT ($p = 0.030$) improvements with Cerebrolysin Follow-up: superiority of Cerebrolysin in SKT performance (week 6: $p = 0.045$; week 9: $p = 0.024$)	[192]
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	End point: day 7 Follow-up: 6–9 months post-TBI		Cerebrolysin-treated patients (GOS: $p < 0.05$)	
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Fei and	Severe–acute TBI	Daily iv. infusions of	End point: significant treatment	[194]

Shenyui (1992)	(GCS: 5–8) Cerebrolysin (n = 20), basic therapy (n = 20) End point: day 7 Follow-up: 6–9 months post-TBI	Cerebrolysin 10 ml add-on to basic therapy vs basic therapy alone for 7 days Start within 24 h after TBI	differences for the reduction of MDA levels ($p < 0.05$) and the GCS improvement ($p < 0.05$) Follow-up: better outcome in Cerebrolysin-treated patients (GOS: $p < 0.05$)	
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Studies with Cerebrolysin in postacute TBI patients are more heterogeneous. When add-on Cerebrolysin therapy (10 ml, intravenous infusions, twice/day) was initiated after acute hospital discharge and maintained for 3 weeks, a faster improvement of some specific motor abilities ($p < 0.001$) was observed during early rehabilitation in a population of TBI patients older than 60 years on average, but no significant changes were recorded in Functional Independence Measure scores.[198] Improvements of cognitive performance induced by Cerebrolysin (30 ml/day, 20 intravenous infusions over 4 weeks) in postacute TBI patients[167,168] were similar (3–4 points in the Syndrom-Kurz test) to that reported in an acute study using the same psychometric instrument.[192] Postacute studies also demonstrated that Cerebrolysin reduces the amount of slow EEG activity, and that this reduction was associated with improved cognition, but not with TBI severity and progression time.[167,168] The positive effects of Cerebrolysin on EEG activity are consistent with the significant improvement of cerebral perfusion pressure induced by the drug in acute TBI patients.[196] Another beneficial action of Cerebrolysin on biological parameters was the significant reduction of the increased blood levels of malondialdehyde (reactive oxygen species generated by lipid peroxidation and biomarker of oxidative stress) in severe–acute TBI patients.[194] As no effect was observed on the activity of the antioxidant enzyme superoxide dismutase, it seems that Cerebrolysin may instead be involved in preventing the formation, rather than in enhancing the elimination, of free radicals.[194]

Results of these preliminary studies indicated that treatment with Cerebrolysin is associated with a faster clinical recovery of general (consciousness and severity), functional (cognitive and motor performance) and biological parameters (EEG activity, cerebral perfusion and oxidative stress) in TBI patients, which may result in a shorter hospitalization time and a better long-term outcome. Further validation of these promising findings in confirmatory RCTs is warranted. Finally, and according to data available, there are no safety constrains for the use of Cerebrolysin at daily doses of 10–60 ml in TBI patients.

Other Clinical Studies With Neuropeptides in TBI Patients

Progesterone, GH, IGF-1 and EPO are the most salient examples of other approaches to the treatment of TBI with peptidergic compounds.

Progesterone

Progesterone is a pleiotropic agent with neuroprotective properties, reducing brain edema, neuronal loss and behavioral deficits in experimental TBI.[79,80,83–85] Preliminary clinical data are encouraging and support the development of progesterone as a future therapeutic option for TBI.

The effects of progesterone in TBI patients were evaluated in two pilot Phase II clinical trials.[199,200] Patients with moderate–severe TBI (GCS: 4–12) who arrived within 11 h of injury were included in the ProTECT trial[199] in which 77 patients were randomized to receive progesterone and 23 patients received placebo. Patients randomized to progesterone had a lower 30-day mortality rate than controls (rate ratio: 0.43; 95% CI: 0.18–0.99). According to the dichotomized GOS-Extended 30 days postinjury, moderate TBI survivors who received progesterone were more likely to have a moderate-to-good outcome than those receiving placebo. No serious adverse events were attributed to progesterone in this trial. In a second study, 159 patients with severe TBI (GCS: ≤8) were enrolled within 8 h of trauma and assigned to receive progesterone (n = 82) or placebo (n = 77).[200] Compared with placebo, patients treated with progesterone had a lower mortality rate at 6-month follow-up ($p < 0.05$) and more favorable outcomes after 3 and 6 months of treatment, as measured by the dichotomized GOS ($p = 0.034$ and $p = 0.048$, respectively) and the modified Functional Independence Measure score ($p < 0.05$ and $p < 0.01$). Again, no adverse events associated with the administration of progesterone were found. These positive results need to be confirmed by ongoing Phase III clinical trials.

GH & IGF-1

GH and IGF-1 contribute to TBI pathophysiology and have attracted some therapeutic interest, particularly in postacute TBI patients with GH deficiency. Exploratory studies have demonstrated beneficial effects after treatment with GH or the combination of IGF-1 and GH in TBI patients.[201–204] Compared with placebo, treatment with combined IGF-1/GH therapy for 14 days produced sustained improvements in metabolic and nutritional end points (higher serum glucose concentrations and a positive nitrogen balance) in moderate-to-severe TBI patients enrolled into a RCT within 72 h of injury.[201] In patients with GH deficiency or insufficiency included in a RCT several years after injury, 1-year GH replacement therapy was superior to placebo in improving cognitive performance in tasks evaluating motor speed, information processing speed, memory and executive functioning.[202] A significant improvement in the quality of life and a normalization of IGF-1 levels were also found in GH-deficient TBI patients receiving GH replacement for 1 year.[203] In a recent open-label study, GH-deficient TBI patients treated with GH and cognitive rehabilitation for 3 months demonstrated a recovery of normal plasma IGF-1 levels, and achieved significantly greater improvements in cognitive parameters (similarities, vocabulary, verbal and total intelligence quotient) than TBI patients without GH deficiency treated with placebo and cognitive therapy.[204] All these data suggest that elevations of peripheral IGF-1 levels may result in improved metabolic and cognitive functions in TBI patients. As there is evidence linking high circulating IGF-1 levels with cancer risk, this therapeutic approach has to be carefully evaluated.[205] The development of IGF-1 analogs could probably offer some advantage to overcome this risk.[44]

Erythropoietin

EPO acts as a growth factor with pleiotropic effects in multiple brain cells and tends to be upregulated in TBI.[91,106,135] As shown in experimental studies, EPO induces anti-apoptotic, anti-oxidant and anti-inflammatory responses in neurons, glial and cerebrovascular endothelial cells, stimulates angiogenesis and neurogenesis and improves structural and functional recovery after brain injury.[135,206,207]

Clinical data available, although inconclusive, suggest that EPO may reduce mortality in TBI patients. In a large RCT with critically ill patients, not specifically designed for TBI, Corwin *et al.* reported a lower mortality in trauma patients treated with epoetin-α (recombinant human EPO) than in those receiving placebo, at both day 29 (adjusted hazard ratio [HR]: 0.37; 95% CI: 0.19–0.72) and day 140 (adjusted HR: 0.40; 95% CI: 0.23–0.69).[208] Treatment with epoetin-α was associated with an increase in the incidence of thrombotic events (HR: 1.41; 95% CI: 1.06–1.86) in this study. A lower TBI mortality was also reported in two studies

using an erythropoiesis stimulating agent (ESA). In a retrospective matched case–control study, severe TBI patients treated with ESA had a significantly lower in-hospital mortality compared with matched patients who did not receive ESA (7.9 vs 24.2%, respectively; odds ratio: 0.27; 95% CI: 0.12–10.62; p = 0.001).[209] Comparing patients who received ESA within 30 days after admission with patients who did not receive it (75 matched pairs) in a prospective observational study, the same investigators reported similar changes in mean GCS, lower in-hospital mortality (9.3 vs 25.3%; odds ratio: 0.25; 95% CI: 0.08–00.75; p = 0.012) and longer intensive care unit stay (16.1 vs 8.6 days; p < 0.001) for ESA-treated patients.[210] Finally, in TBI patients randomized to EPO (n = 11) or placebo (n = 5) within 6 h of injury, EPO did not induce significant changes compared with placebo in the levels of neuronal cell death markers (S100B and Neuron Specific Enolase), mean maximum intracranial pressure or length of hospital stay.[211] RCTs are required to evaluate the efficacy of EPO in TBI treatment.

Future Perspective & Conclusion

Scientific evidence accumulated during the past decades on TBI pathophysiology and therapy supports the development of multimodal drugs for TBI treatment. It is assumed that all drugs targeting a single TBI pathological factor failed till now probably because the pathogenesis of TBI involves multiple cellular and molecular processes. Therefore, multifunctional drugs acting on several TBI pathophysiological pathways seem to represent a better therapeutic alternative in TBI. Since peptide-mediated mechanisms were found to contribute to most of the processes of secondary brain damage and neurorepair after TBI, multimodal peptidergic drugs may be a good option for the effective treatment of TBI patients.

Preliminary clinical results obtained with the peptidergic drug Cerebrolysin, as well as with the neuropeptides progesterone and EPO, are very promising and support the pertinence of the approach to TBI therapy with multimodal peptidergic agents. Treatment with Cerebrolysin was associated with a faster clinical recovery, a shorter hospitalization time and a better long-term outcome in TBI patients. However, data about the effects of Cerebrolysin on survival after TBI are lacking. Progesterone therapy had some advantages over placebo on TBI mortality and clinical outcome at certain follow-up time points; whereas EPO seems to reduce in-hospital mortality without affecting clinical TBI outcome. All these positive findings still require validation by confirmatory RCTs.

At present, there is no effective drug therapy approved for TBI. Thus, the main priority now is to try to confirm the promising results previously obtained with Cerebrolysin and progesterone in ongoing Phase III RCTs. Important issues to be further addressed in TBI treatment include the improvement of the poor acute outcome in elderly patients, the implementation of the more efficient drug strategies for the prevention, recovery and management of long-term disabilities, and the identification of the most appropriate time windows for therapeutic interventions during acute and postacute TBI phases. According to current preclinical and clinical levels of evidence, peptidergic drugs with pleiotropic neuroprotective effects and multimodal neuroregenerative activity are the most promising therapeutic candidates to improve acute outcome and long-term recovery after TBI.

Executive Summary

- Traumatic brain injury (TBI) affects more than 10 million people annually worldwide and is associated with high rates of hospitalization, mortality and disability. Although TBI survival improved continuously for decades, particularly in developing countries, implementation of an effective drug therapy for TBI represents an unmet clinical need.

- Traumatic brain damage involves multiple pathologic processes underlying the primary and secondary mechanisms of injury. These pathologic processes are amenable to therapeutic interventions for brain protection and repair intended to prevent secondary lesions.

- Peptidergic-mediated mechanisms are involved in neurodegeneration and neurorepair after TBI:

    - Neuroinflammation (microglia activation and increased production of proinflammatory cytokines);
    - Endocrine dysfunctions (deficits of gonadal steroids, growth hormone [GH] and IGF-1);
    - Alterations of neurotrophic factors (reduced brain-derived neurotrophic factor and NGF activity, and upregulation of VEGF);
    - Abnormal processing of Alzheimer's disease-related proteins (increased accumulation of amyloid-β and tau proteins);
    - Neuropeptides may contribute to the enhancement of neuronal survival and functional recovery after TBI by inducing Akt activation and GSK-3β inhibition through the PI3K/Akt/GSK-3β signaling pathway.

- Neuropeptides act on multiple TBI pathophysiological pathways and are good candidates for multimodal treatment in neurotrauma. To date, all drugs targeting a single TBI pathological pathway failed to show clinical efficacy, probably because TBI pathophysiology involves multiple cellular and molecular mechanisms of secondary brain damage.
- Peptidergic drugs represent a multimodal therapy alternative to improve acute outcome and long-term recovery in TBI patients:
    - Cerebrolysin ® (Ever Neuro Pharma GmbH, Unterach, Austria) is a multimodal peptidergic drug demonstrating neuroprotective and neurorestorative properties;
    - Treatment with Cerebrolysin resulted in a faster clinical recovery, a shorter hospitalization time and a better long-term outcome in TBI patients.
- Clinical TBI studies were also conducted with other neuropeptides (progesterone, GH, IGF-1 and erythropoietin):
    - Treatment with progesterone showed advantages over placebo regarding TBI mortality and clinical outcome;
    - Exploratory studies demonstrated beneficial effects after treatment with GH, or the combination of IGF-1 and GH, in postacute TBI patients, which supports the development of IGF-1 analogs;
    - Erythropoietin reduced in-hospital mortality with no effects on TBI outcome.
- Further validation of these promising findings in confirmatory randomized-controlled clinical trials is warranted. According to current preclinical and clinical evidence, multimodal peptidergic drugs constitute a therapeutic option for the effective treatment of TBI patients.

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Papers of special note have been highlighted as:

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Future Neurology. 2013;8(2):175-192.