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REVIEW ARTICLE

Neuropharmacology in traumatic brain injury: from preclinical to clinical neuroprotection?

Dominique Lerouet, Catherine Marchand-Leroux, Valérie C. Besson

UMR-S1144 - Optimisation Thérapeutique en Neuropsychopharmacologie, Faculté de Pharmacie de Paris, Université de Paris, Paris, France

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*Correspondence and reprints:
valerie.besson@u-paris.fr

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ABSTRACT

Traumatic brain injury (TBI) constitutes a major health problem worldwide and is a leading cause of death and disability in individuals, contributing to devastating socioeconomic consequences. Despite numerous promising pharmacological strategies reported as neuroprotective in preclinical studies, the translation to clinical trials always failed, albeit the great diversity of therapeutic targets evaluated. In this review, first, we described epidemiologic features, causes, and primary and secondary injuries of TBI. Second, we outlined the current literature on animal models of TBI, and we described their goals, their advantages and disadvantages according to the species used, the type of injury induced, and their clinical relevance. Third, we defined the concept of neuroprotection and discussed its evolution. We also identified the reasons that might explain the failure of clinical translation. Then, we reviewed post-TBI neuroprotective treatments with a focus on the following pleiotropic drugs, considered "low hanging fruit" with high probability of success: glitazones, glibenclamide, statins, erythropoietin, and progesterone, that were largely tested and demonstrated efficient in preclinical models of TBI. Finally, our review stresses the need to establish a close cooperation between basic researchers and clinicians to ensure the best clinical translation for neuroprotective strategies for TBI.

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of death and disability among youths in industrialized societies that imposes a substantial social and economic burden on the community [1,2]. It is prevalent in both low- and high-income countries and affects people of all ages.

Traumatic brain injury is defined as damage to the brain sustained after the application of external physical force that causes temporary or permanent functional or structural brain damages. The latter can be mild, moderate, or severe [3,4].

Each year, more than 50 million new TBI cases occur in the world, and over 90% are mild TBI. Indeed, according to the Glasgow Coma Scale (GCS), the most widely used measure of TBI severity, TBI can be range from mild (including concussion; GSC 13–15) to moderate (GSC 9–12) and severe TBI (GSC 3–8) [5]. Because by definition mild TBI is non-fatal and should not alter life expectancy, prolonged symptoms can lead to lifelong disabilities [3]. Therefore, the concept of "mild TBI" can be misleading, as it does not reflect the time course of injury symptoms and long-term effects. Although the incidence of severe TBI is less important, about 40% of patients will die from their injury, and 60% will have an unfavorable outcome [6]. It is predicted that close to 50% of the world's population will sustain at least one TBI in their lifetime.

Traumatic brain injury may originate from road traffic incidents, falls, sports, military conflicts, and more

recently from terrorism. A recent systematic review and meta-analyses of articles describing the epidemiology of TBI in 16 European countries showed that the first two causes were the most frequent, with falls being reported more frequently than motor vehicle accidents [7]. However, within the studies that mainly focus on more severe TBI, the latter remains dominant as a cause of injury. Moreover, a correlation was found between the cause of injury and age, with falls being most common in elderly and children subpopulations, while road traffic incidents were the most frequent in young adults.

Reported new TBI cases vary greatly between countries and regions. In low- and middle-income countries, the rising burden of TBI due to the increase of road traffic incidents mainly affects youths, while in high-income countries the changing epidemiology of TBI is related to a high and increasing incidence in infants and elderly people. At last, sports, such as rugby and boxing, and military conflicts also enhance the number of TBI.

Regardless of age, TBI represents 30–40% of all injury-related deaths. Moreover, it is now clear that TBI is an important risk factor for the later development of dementia and neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease [4,8], while repetitive mild TBI results in a distinct pathology called chronic traumatic encephalopathy (CTE) [9]. Moreover, unlike other neurodegenerative diseases, the development of CTE symptoms often occurs earlier in life. Unfortunately, currently, CTE can only be diagnosed postmortem [10]. Epilepsy is also a well-recognized complication of TBI, increasing with the severity of the latter. Thus, TBI accounts for about 5% epilepsy cases in the entire population [1]. Altogether, these data reinforce the view that TBI can evolve into a progressive lifelong illness.

Traumatic brain injury represents a dynamic and complex pathophysiological disease characterized by primary damages leading to secondary lesions. Unfortunately, to date, there is still no protective pharmacological treatment. Pinpointing the pathophysiological mechanisms at the cellular and molecular level is a key step for the identification of pharmacological targets. The interplay between pharmacological agents and their biological targets produces directly or indirectly quantifiable effects. Identification and quantification of these effects in animal models and in humans represent the objectives of preclinical and clinical pharmacology.

Traumatic brain injury is considered as a silent epidemic present throughout the world, as the problems resulting from TBI are often not immediately visible. Each year, up to 50 million new cases occur worldwide [1,11] and about 2.5 million in Europe [1], causing more than 50 000 deaths annually in the United States and Europe [1,12,13]. TBI incidence increases with age, as well as the outcome severity. In high-income countries, TBI remains the main cause of death in children and adolescents, and affects more boys than girls whatever the age range [1]. Although 65 years old people represent only 10% of TBI cases, they account for 50% of TBI-related 10-year mortality risk. The epidemiology of TBI has changed over the years, as a clear shift toward older traumatized patients has been observed in high-income countries, with falls representing the primary cause of TBI in elderly population [14]. Unsurprisingly, older adults experience poorer outcomes than younger adults with similar TBI severity [15]. Studies also showed that prevalence of TBI is higher in males than females, but this gender disparity is reduced at both extremes of age [1].

For over a century, severe TBI-related mortality has dropped by half thanks to advances in medical science and to preventive measures. However, for the past 25 years, no further improvement has been noted [1,16].

Extension and localization of acute and chronic traumatic brain lesions depend on the severity and type of TBI. Briefly, the pathophysiology of TBI involves both primary and secondary injury. Primary injury that is directly caused by mechanical pulse/force can be induced by numerous mechanisms, such as brain contusion, hematoma, shearing, and stretching of the brain tissue caused by motion of the brain structures relative to the skull. The secondary injury is a consequential event of the primary injury and includes complex biochemical and physiological processes that manifest over a period of hours to days and even months and years. Animal models, commonly used in TBI research, have been developed and used to experimentally mimic some aspects of the behavioral, tissular, and cellular consequences of human TBI in order to understand post-traumatic pathophysiology. Pharmacological studies have been performed to identify cellular and molecular targets promoting the reduction of acute and chronic brain lesions.

ANIMAL MODELS OF TBI

Goals of preclinical TBI models are to identify biomarkers and understand mechanisms involved in post-

traumatic consequences in order to propose therapeutic strategies for TBI patients. Animal models of TBI are an essential step toward a better understanding of the pathophysiology of this pathology and the development of novel therapies. The failure of bench to bedside translation might be overcome by modifying several preclinical practices. In particular, to increase translation potential of preclinical studies, an important consideration should be to include more testing in females, as the susceptibility to injury differs between genders [17]. Moreover, a better diagnosis and treatment requires a better understanding of the injury mechanisms in a well-defined severity of mild, moderate, and severe injury in different models that may potentially reflect the various types of human brain injuries [18]. Today, there is no established guideline to assess injury severity in experimental TBI, which makes it challenging for animal model findings to a clinical translation. Indeed, clinical evaluation method, such as the GCS, is inadequate to evaluate the severity of brain injury in animals, as in humans, TBI severity is assessed in verbal response to the state of consciousness. Therefore, over the years, a broad variety of behavioral tests have been developed to assess sensorimotor and cognitive functions, social interactions, and anxiety-like and depression-like behavior in animal models of neurological diseases [19–22]. As for animal models, there is no perfect behavioral test and the most suitable one has to be chosen depending on the objectives of the research project.

The classification of TBI severity in experimental models also varies with the device model used, and from one laboratory to another. This highlights the need to define convergent standard criteria for mild, moderate, and severe TBI in animal models. The heterogeneity of human TBI makes it difficult to develop a single animal model that can accurately replicate all of the primary and secondary events observed in humans. Therefore, since the 1940s, numerous animal models of TBI have been developed according to the presence or absence of craniectomy before brain trauma and the type of force applied (*Table I*). Both present advantages and disadvantages, head injuries are typically classified as closed or penetrating. The first one is usually used to describe road traffic incidents, assaults, and falls, while the second one results from gunshots or stab wounds. More recently, military conflicts have generated a third category known as blast injury due to explosive devices.

Animal models can also be classified into focal, diffuse, and mixed (focal and diffuse) injury [18]. Focal brain injury, defined as a localized tissue damage, results from a blow to the head, road traffic incidents or assaults, while diffuse brain injury is caused by acceleration or deceleration impact, such as after blast injury. At last, mixed injury results from falls or sport injury.

So far, for ethical, economical, and practical reasons, rodents, that is, mice and rats, are currently the mostly used animals in TBI studies, but experiments in larger animals (cat, rabbit, dog, sheep, ferret, swine, non-human primate) also exist and play crucial role in understanding the underlying mechanisms of TBI, given their neuroanatomical similarities with the human brain [29]. The lack of effective pharmacological treatment for TBI may be partly ascribed to the predominant use of rodents with lissencephalic brain as opposed to larger species with gyrencephalic brain (sheep, pig and non-human primate) and higher white to gray matter ratio close to human brain [30,32]. Indeed, lissencephalic brain will experience less brain deformation than gyrencephalic one, given that gyri influence the movement of the brain within the skull. Therefore, the application of the same acceleration force will induce only minor injury in animals with lissencephalic brains compared to large animals. Moreover, the sensitivity of gray and white matter tissue to injury is different, as the latter appears more vulnerable. Thus, the use of large animal species may enhance successful translation to clinic given the similarities in neuroanatomical structure to the human brain and a closer resemblance to the pathophysiological and clinical manifestations of TBI in humans when compared with rodent models [32]. Another advantage of large animal species is that their brains lend themselves to superior quality MRI studies, by using, in addition, the same instrumentation for physiological monitoring than in humans, with similar values observed, facilitating therefore the clinical translation. Now, it remains to establish which of these large animal species would be the more suitable. Non-human primates might be the candidate of choice as they also express distinct cognitive and behavioral superiority, advantageous for neurobehavioral testing.

Nevertheless, mice are still increasingly being used due to the availability of transgenic lines.

All models are valid whether their limitations are taken into account when interpreting the results. For example, the necessity of craniectomy and the use of anesthetic and/or analgesic can modify and alter TBI outcomes. Moreover, of note, models with craniectomy

Table I Animal models of TBI [23–31]

Model	Animal		Type of injury	Clinical relevance	Advantages & disadvantages
	Rodent	Large animal			
With craniectomy					
Controlled cortical impact	X	X	Mixed (focal and diffuse)	Sport-related TBI	+: fine tuning of injury severity [controlled depth, velocity, impact (dwell) time, size and type of impactor tip]; no contrecoup injury; no skull fracture −: craniectomy
Fluid percussion	X	X	Focal, diffuse, or mixed	Sport-related TBI	+: unique or repeated; central or lateral impact; fine tuning of injury severity; highly reproducible; no skull fracture −: craniectomy
Weight drop	X	Not reported	Focal, diffuse, or mixed	Falls and motor vehicle accidents	+: tuning of injury severity (mass of the weight and the height from which it falls); unique or repeated; no skull fracture −: hardly reproducible; variable mortality rate; risk of contrecoup injury; craniectomy
Without craniectomy					
Controlled cortical impact	X	X	Mixed	Sport-related TBI	+: fine tuning of injury severity [controlled depth, velocity, impact (dwell) time, size and type of impactor tip]; no contrecoup injury −: risk of skull fracture
Weight drop	X	Not reported	Focal, diffuse, or mixed	Falls and motor vehicle accidents	+: fast; tuning of injury severity (mass of weight and the height from which it falls); unique or repeated −: hardly reproducible; high variability of mortality; risk of skull fracture; risk of contrecoup injury
Impact acceleration	X	X	Mixed	Falls and motor vehicle accidents	+: unique and repeated −: high variability of injury severity
Inertial acceleration	Not reported	X	Diffuse	Motor vehicle accidents	+: pure diffuse injury
Blast injury model	X	X	Diffuse	Civilian and military-related TBI	+: pure diffuse injury

do not reflect the chronology of events occurring in the majority of TBI, such as falls or road traffic accidents. Indeed, in humans, the first TBI event is the impact on the skull, eventually followed by a skull fracture or opening, with or without penetration, while in animal models with craniectomy, the first event is the opening of the skull followed by the impact on the brain, but without penetration. In addition, the craniectomy prevents the intracranial pressure increase that is an important consequence of severe TBI, requiring a craniectomy afterward. Therefore, these models are not the most relevant.

Moreover, to date, there is no doubt that a strong correlation exists between the years of exposure to repetitive mild TBI and the incidence of CTE. The latter is typical of the contact forces that occur to athletes on the field of play, and, more recently, has also been reported in military personnel exposed to explosive devices. Although long-term consequences of repetitive mild TBI have long been considered as insignificant, the last decade has seen increased awareness of potential chronic neurodegenerative detriment from such injuries. In this context, several models have been developed to reproduce repetitive mild TBI in animals,

in order to identify appropriate therapeutic strategies [9,31,33-35].

Given the heterogeneity and etiological complexity of TBI, no single animal model can mimic all TBI consequences. Therefore, the recent preclinical strategy is to perform large-scale preclinical multicenter consortium involving multiple TBI models to ensure replication of findings and the discovery of new treatments. This approach has been developed by few groups [36]. Moreover, it seems crucial to set up a new consciousness that any potential pharmacological strategy having been proved efficient for TBI in rodent models needs to be further investigated in large animal TBI models.

Animal models are also extremely valuable to discover biomarkers that could diagnose TBI, predict its outcomes and monitor its evolution, and evaluate the therapeutic efficiency of new therapies. Thus, a recent review reports several tools, such as biofluid biomarkers, that have been explored in both preclinical and clinical TBI studies, and that have shown potential in diagnosis, prognosis or monitoring [37].

NEUROPROTECTION

The concept of neuroprotection

Pharmacological protection has been firstly introduced in 1980 in the field of excitotoxicity and cerebral ischemia, and has been secondly extended to other acute and chronic brain pathologies. Three scenarios can be observed after brain injury: (i) neuron remains intact without sequelae, (ii) neuron is suffering, or (iii) neuron is dying. The last two situations require neuroprotective agents. Neuroprotection has been defined as the protection or preservation of neuronal structure and function. However, this definition, originated from acute and chronic neurodegenerative disorders, is quite broad and "neuronocentric." Thus, clinicians proposed a more pragmatic definition, consisting in the maintenance of neuronal and glial damage without any clinical symptoms. Indeed, increasing evidence underscores the importance of glial (astrocyte, microglia, oligodendrocyte) and endothelial cells which have complex and related interplay. Thus, the promotion of recovery and optimal function of glial and endothelial cells is associated with the inhibition of neuronal cell death.

Although TBI affects all brain cells in both gray and white matters, researchers mainly focused on neuronal death for many years. Today, it is well established that protective strategies have to target not only neurons, the noble but the more vulnerable of central nervous system (CNS) cells, but also other cell types such as glial and vascular cells (endothelial cells, smooth muscle cells, and pericytes) to promote neuroprotection. In the field of TBI, many molecules have been tested in animal models and have proven their neuroprotective activities leading to their clinical evaluation. Despite the numerous promising candidate therapies identified using preclinical TBI models, none has yet successfully shown clinical benefits. Several reasons have emerged from the retrospective analysis of preclinical and clinical studies:

- huge interspecies differences (between rodents and humans) in anatomy, morphology, metabolism, neurobiology, and life span
- differences in size and cell density in rodent *versus* human brain
- lissencephalic brain for rodents (main experimental studies) *versus* gyrencephalic brain for humans [30,32]
- less white matter in rodents (10% white matter and 90% gray matter) than in humans (50% white matter and 50% gray matter)
- irrelevant animal models of TBI
- inadequate sample size
- preclinical models performed mainly in males [17]
- lack of experimental studies taken into account comorbidities
- poor understanding of secondary lesions, such as white matter injury and long-term behavioral consequences
- mild TBI 80% versus severe–moderate TBI 20%
- inadequate and untranslatable behavioral outcomes
- use of anesthesia in animal models with potential drug/anesthetic interactions and/or neuroprotective effects
- inappropriate therapeutic window [38]
- lack of pharmacokinetic/pharmacodynamic (PK/PD) modeling studies: the concentration–response and time–response relationships, the plasma protein binding, the influence of metabolism, and the measurements of metabolites (actives or inactives) provide valuable informations for translational purposes
- lack of predictive biomarkers
- no correlation between severity of TBI animal models and that of traumatized patients included in clinical trials
- heterogeneity of TBI patients *versus* reproducible animal models
- lack of multicenter preclinical trial of neuroprotective molecules

Nonetheless, these failures can be addressed with improved preclinical drugs testing in TBI models. A TBI preclinical consortium, Operation Brain Trauma Therapy, has emerged in the United States to perform multicenter preclinical studies [36]. The objective is to identify the most promising therapies that could demonstrate robust beneficial effects across TBI models and those with model dependent effects to guide to patient with specific anatomical TBI phenotypes. This consortium, which has already evaluated ten therapies and assessed three serum biomarkers, links the finding of optimized preclinical studies to clinical trial design to produce successes in therapy and biomarker development in TBI.

PHARMACOLOGICAL STRATEGIES

Traumatic brain injury is a complex process that results from primary and secondary injuries. The latter can happen from minutes to months from the primary impact and consists of a cascade of events responsible for further brain damages [2,5,39]. Indeed, different cell death mechanisms drive TBI, such as excitotoxicity that is characterized by the release of neurotransmitters like glutamate [40], leading to increased intracellular calcium. In turn, high intracellular calcium concentration activates an array of catabolic enzymes including endonucleases, proteases, and phospholipases, which damages DNA, structures, and membranes. Another prominent mechanism that happens shortly after TBI is oxidative stress, due to excessive production of both reactive oxygen and nitrogen species (ROS and RNS) that cause lipid peroxidation, protein carbonylation and DNA oxidation. Neuroinflammation plays also a key role in post-traumatic brain injury. Thus, inflammatory responses are activated with the invasion of monocytes, neutrophils, and lymphocytes through the blood–brain barrier (BBB), as well as via the glial cells, especially microglial cells that release proinflammatory cytokines but also produce ROS. Although all these post-TBI mechanisms occur in both animals and humans, they may differ in terms of timing, intensity and duration, according to the age of TBI, the TBI severity, and the brain lesion location.

Consequently, many preclinical and clinical studies have tested the therapeutic efficacy of drugs targeting these mechanisms, including, among others, excitatory amino acid inhibitors, calcium channel blockers, free radical scavengers and antiinflammatory strategies. Interested readers can find results of these studies in some reviews that need not to be reitered here [5,41–46]. Regarding past, present, and future clinical trials, about 150 drugs were or are going to be evaluated in 447 clinical trials (https://www.clinicaltrials.gov/ct2/results?term=drug&cond=Traumatic+Brain+Injury&age_v=&gndr=&type=Intr&rslt=&Search=Apply).

The distribution per age group and study phase are presented in *Table II*. However, among all these drugs, each time that a neuroprotective one was put to test in phase III trials, it consistently failed to make a significant impact on day-to-day clinical practice.

Considering the interrelationship between all the different cells mentioned above, the ideal therapeutic strategy should therefore target the "neurogliovascular" unit. Moreover, as numerous molecular mechanisms contribute to the complexity of TBI, drugs targeting only one mechanism failed to protect the whole unit. Thus, pleiotropic drugs controlling multiple deleterious biochemical pathways demonstrated beneficial effects in experimental studies and showed promise for success in clinical trials. Therefore, we limited this review to the following pleiotropic strategies, glitazones, glibenclamide, statins, erythropoietin (EPO), and progesterone, that were largely tested in preclinical models of TBI (*Table III*). In addition, we focused on pharmacological therapies that are considered "low hanging fruit," that is, therapies approved by agencies, such as European Medicine Agency (EMA) or Food and Drug Administration (FDA), for other indications that should have a high probability of success. Moreover, these pleiotropic therapies have been translated from bench to bedside, except glitazones. Consequently, their side effects being well known, it is easier to evaluate the benefit/risk balance of these drugs.

Glitazones, also called thiazolidinediones, are synthetic peroxisome proliferator-activated receptor (PPAR) γ agonists prescribed worldwide to treat hyperglycemia and diabetes. PPAR, or nuclear receptor 1 C (NR1C), family are nuclear receptors leading to the regulation of gene transcription essential in metabolic processes and cell differentiation. Three PPAR isotypes are currently identified in mammals: PPARα (NR1C1), β/δ (NR1C2), and γ (NR1C3). All subtypes are expressed in the CNS, albeit at different levels [132]. Under physiological conditions, PPARγ is mainly expressed in neuronal cells and astrocytes [133,134], while its expression is increased in microglia during inflammatory conditions [134]. Most of PPARγ agonists activate PPARγ, as well as other PPAR, but are also involved in PPAR-independent pathways. Thus,

Table II Clinical trials in TBI.

	Study phase				
Age group	I	II	III	IV	% of study per age group
Child (birth-17)	2%	5%	4%	2%	13%
Adult (18–64)	9%	21%	11%	9%	50%
Older adult (65+)	5%	15%	10%	7%	37%
% of study per phase	16%	41%	25%	18%	100%

glitazones exert pleiotropic effects through PPAR-dependent and -independent mechanisms that are not fully differentiated. A large body of preclinical data supports the future translation to clinical trial for TBI (*Table III*).

Glibenclamide, also known as glyburide, is an antagonist of SulfonylUrea Receptor-1-Transient Receptor Potential Melastatin member 4 (SUR1-TRPM4). It is a well-known drug used in type 2 diabetes mellitus to promote the release of insulin by blocking SUR1-pancreatic potassium adenosine triphosphate (ATP) channels. SUR1 is a member of the ATP binding cassette transporter superfamily also coupled to TRPM4. This ion channel complex is a hetero-octameric structure comprising four SUR1 subunits and four subunits of TRPM4. SUR1-TRPM4 is not normally present in the brain but undergoes upregulation in multiple CNS cell types after TBI (neurons, microglia, astrocytes, endothelial cells) [54,55,60,135]. Glibenclamide has received considerable attention due to the demonstration of its pleiotropic protective effects in animal models of TBI (*Table III*), which has led to a phase II clinical trial currently evaluating whether glibenclamide will decrease post-traumatic edema and/or hemorrhage compared to placebo (NCT01454154; https://clinicaltrials.gov/ct2/show/NCT01454154).

Statins are a well-known class of medications acting through the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase to decrease hepatic cholesterol synthesis, which in turn lowers serum low-density lipoprotein levels by hepatic low-density lipoprotein–receptor upregulation. Studies have identified the pleiotropic properties of statins and their beneficial effects in other pathologies than hyperlipidemia, such as TBI. Modulation of lipid synthesis, by inhibition of HMGCoA reductase, lowers mevalonic acid levels promoting numerous intracellular signaling cascades, which leads to the pleiotropic effects of statins (*Table III*). A clinical study demonstrated that prior statin usage to TBI did not improve functional outcome 3 months after TBI [136,137] but improved functional recovery 12 months post-injury [136]. However, cardiovascular comorbidities caused the benefit loss of premorbid statin use. In addition, post-TBI treatment with atorvastatin reduced tumor necrosis alpha (TNFα) and improved functional outcome [138]. A randomized double-blind placebo-controlled clinical trial, enrolling 65 TBI patients, demonstrated an improved functional outcome at 3 months, but without reducing contusion [139]. These results should be followed by larger multicenter clinical trials.

EPO is a member of the type 1 cytokine superfamily, produced by kidneys, leading to the production of erythrocytes. The role of EPO goes far beyond erythropoiesis, as EPO receptor (EPOR) is expressed in erythroid tissue but also in non-erythroid tissue, such as brain. EPOR is a transmembrane receptor with tyrosine kinase activity by Janus kinase-2 (JAK-2). Brain-expressed EPOR is structurally different from that of erythroid tissue. Indeed, it is a heterodimer composed of a monomer of the canonical EPOR and another subunit of the ß-common receptor, cluster of differentiation (CD) 131 (EPOR-ß-comm) that is identical to the β region of cytokine receptor, such as interleukin (IL) 3 or granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors. EPOR activation, by EPO or its carbamylated analogs, leads to an intracellular signaling cascade resulting in neuroprotective effects (*Table III*). Results from a double-blind randomized controlled trial in patients with TBI showed no evidence of EPO efficiency neither on the neurological outcome at 6 months [140] nor on the number of patients with severe neurological dysfunction (EPO-TBI) [141]. However, recently, a follow-up of patients treated in EPO-TBI trial [141] will enable to evaluate any possible long-term differences (survival, neurological function

Table III Preclinical studies of pleiotropic strategies in TBI.

Pleiotropic strategy	Models & species	Cellular effects	Molecular effects	Effects on post-traumatic consequences	Ref.
Peroxisome Proliferator-Activated Receptor (PPAR) γ agonists (rosiglitazone, pioglitazone)	Models: FPI CCI Species: rodents	↓axonal injury ↓ apoptosis ↓ autophagy ↓ microglial activation ↑ M2 microglia phenotype ↑ neuron survival	mRNA: ↓ TNFα, IL6, MCP1, ICAM1, caspase-3, Bax ↑ HSP27, HSP70, HSP32/HO-1, Cu/Zn-SOD, MnSOD → MMP9, iNOS, COX2 Protein: ↓ ICAM1, mitochondrial dysfunction (MitoNEET) ↓ or →TNFα, IL6 → IL1, iNOS, GLT-1 ↑ HSP27, HSP32/HO-1 Activity: ↑ PPAR alpha activity	↓sensorimotor and motor coordination deficits ↓ or →spatial memory deficit ↓ or →brain lesion	[47–53]
Glibenclamide	Models: CCI FPI PBBI Species: Rodents	↓apoptosis ↓ neurodegeneration	Protein: ↓ ZO-1 loss, occludin loss	↓motor deficit and spatial learning and memory deficits ↓ blood extravasation, BBB disruption and edema ↓ brain lesion	[36,54–60]
Statins (atorvastatin, simvastatin, lovastatin)	Models: CCI FPI PBBI Species: rodents	↓neuronal cell death ↓ apoptosis ↓ neurodegeneration ↓ microglial activation ↓ astrogliosis ↑ neuron survival ↑ synaptogenesis ↑ angiogenesis ↑ cerebral blood flow	mRNA: ↓ COX2, IL18, IL1ra Protein: ↓ TNFα, IL1β, IL6, eNOS, ICAM1, TLR4, NF-κB, Aβ ↑ VEGF, BDNF Lipid: ↓ cortical cholesterol	↓sensorimotor and motor coordination deficits ↓ or ↑ spatial memory ↓ or ↑ brain lesion ↓ edema	[61–79]
Progesterone	Models: CCI FPI WDI Species: Rodents	↓axonal injury ↓ astrogliosis ↓ microglial activation ↓ neutrophil activation ↓ apoptosis ↑ or →neuron survival ↑ vessel density ↑ circulating endothelial progenitor cells	mRNA: ↓ IL1β, TNFα, TLR2, TLR4, Bax, Bad ↑ Bcl-2, Bcl-xL Protein: ↓ IL1β, IL6, TNFα, ICAM-1, NF-κB p65, Bax, Bad ↑ Bcl-2, Bcl-xL ↓ lipid peroxidation	↓sensorimotor, motor coordination and spatial learning and memory deficits ↓ BBB permeability ↓ intracranial pressure ↓ or →edema ↓ or →brain lesion	[80–115]
Erythropoietin	Models: CCI FPI PBBI	↓apoptosis ↓ astrogliosis ↑ neuron survival ↑ neurogenesis	mRNA: ↑ Nrf2 Protein: ↓ IL1β, IL6, MIP-2, S100B, caspase-3, Bax	↓edema ↓ or →brain lesion ↓ or →sensorimotor deficit, spatial learning and memory deficit	[116–131]

Table III. Continued

Pleiotropic strategy	Models & species	Cellular effects	Molecular effects	Effects on post-traumatic consequences	Ref.
	WDI	↑ angiogenesis	↓ or → p-ERK-1		
	Species:	↑ vascular density	↑ or ↓ NO		
	Rodents	↑ cerebral blood flow	↑ VEGF, p-VEGFR2, Bcl-2		
			↑ or → p-Akt		

Abbreviations: Akt, protein kinase B; Aβ, beta-amyloid protein; Bad, Bcl-2-associated death promoter; Bax, Bcl-2-associated X protein; BBB, blood-brain barrier; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma extra-large; BDNF, brain-derived neurotrophic factor; CCI, controlled cortical impact; COX2, cyclooxygenase type 2; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FPI, fluid percussion injury; GLT-1, glutamate transporter 1; HO-1, heme oxygenase-1; HSP, heat shock protein; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; IL1ra, interleukin 1 receptor antagonist; iNOS, inducible nitric oxide synthase; MCP1, monocyte chemoattractant protein 1; MIP-2, macrophage inflammatory protein 2; MMP9, matrix metalloproteinase type 9; NF-κB; NO, nitric oxide; Nrf2, nuclear factor erythroid-2-related factor 2; nuclear factor-kappa B; PBBI, penetrating ballistic-like brain injury; PPAR, peroxisome proliferator-activated receptor; S100B, S100 calcium-binding protein B; SOD, superoxide dismutase; TLR, toll-like receptor; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; WDI, weight drop injury; ZO-1, zonula occludens-1.

and quality of life) between patients treated with EPO or placebo (NCT03061565; https://clinicaltrials.gov/ct2/show/NCT03061565).

Progesterone is a female steroid hormone that easily crosses the BBB, rapidly diffuses throughout the brain and is well tolerated. The biosynthesis of this hormone is a multistep process starting with cholesterol, passing by pregnenolone, progesterone, aldosterone, cortisol, testosterone, and estradiol. These multiple conversions, many bidirectional, in the biosynthesis of steroids reveal that progesterone effects not only come from progesterone itself, but may also be the result of these other related hormonal effects. Indeed, the mechanisms of progesterone administration are multiple, inducing pleiotropic effects. Neuroprotective efficacy of progesterone has been demonstrated in a large number of TBI animal models (*Table III*). However, ProTECT III and SyNAPSe (2000 patients) [142,143], two phase III clinical trials enrolling more than 2 000 patients, failed to show clinical benefit of progesterone treatment.

CONCLUSION

Over the past thirty years, more than 40 major neuroprotective drugs assessed in clinical trials failed in phase II or III [144]. This gap between the bench and the bedside needs to be urgently addressed to improve both patient survival and outcomes. Recommendations have been made for improving successful translation of neuroprotective agents from preclinical to clinical TBI studies [145]. The failure in introducing efficient drugs and clinical protocols shows the need to establish a closer cooperation between basic researchers and clinicians that should help to define more realistic goals and provide a more objective purpose, guiding research protocols in useful directions [146]. In conclusion, the ground for a robust and relevant preclinical evaluation of drugs requires testing in multicenter preclinical trials on different models of TBI to mimic at best the heterogeneity of clinical situations. The decades of clinical translation failure clearly demonstrate that research in neuroprotection is especially difficult but basic researchers and clinicians should not give up as there is still an urgent need for efficient pharmacological intervention for TBI patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ATP – adenosine triphosphate
BBB – blood–brain barrier
CD – cluster of differentiation
CNS – central nervous system
CTE – chronic traumatic encephalopathy
EMA – European Medicine Agency
EPO – erythropoietin
EPOR – erythropoietin receptor
FDA – Food and Drug Administration
GCS – Glasgow Coma Scale
GM-CSF – granulocyte-macrophage colony-stimulating factor
HMGCoA – 3-hydroxy-3-methylglutaryl coenzyme A
IL – interleukin
JAK-2 – Janus kinase-2
NR1C – nuclear receptor 1 C
PK/PD – pharmacokinetic/pharmacodynamic
PPAR – peroxisome proliferator-activated receptor
RNS – reactive nitrogen species
ROS – reactive oxygen species
SUR – sulfonylurea receptor
TBI – traumatic brain injury
TNFα – tumor necrosis factor α
TRPM4 – transient receptor potential melastatin member 4

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