Impact of pediatric traumatic brain injury on hippocampal neurogenesis

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Abstract

Traumatic brain injury (TBI) is a major cause of mortality and morbidity in the pediatric population. With advances in medical care, the mortality rate of pediatric TBI has declined. However, more children and adolescents are living with TBI-related cognitive and emotional impairments, which negatively affects the quality of their life. Adult hippocampal neurogenesis plays an important role in cognition and mood regulation. Alterations in adult hippocampal neurogenesis are associated with a variety of neurological and neurodegenerative diseases, including TBI. Promoting endogenous hippocampal neurogenesis after TBI merits significant attention. However, TBI affects the function of neural stem/progenitor cells in the dentate gyrus of hippocampus, which results in aberrant migration and impaired dendrite development of adult-born neurons. Therefore, a better understanding of adult hippocampal neurogenesis after TBI can facilitate a more successful neuro-restoration of damage in immature brains. Secondary injuries, such as neuroinflammation and oxidative stress, exert a significant impact on hippocampal neurogenesis. Currently, a variety of therapeutic approaches have been proposed for ameliorating secondary TBI injuries. In this review, we discuss the uniqueness of pediatric TBI, adult hippocampal neurogenesis after pediatric TBI, and current efforts that promote neuroprotection to the developing brains, which can be leveraged to facilitate neuroregeneration.

Key Words: adult hippocampal neurogenesis; astrocytes; development; microglia; neuroinflammation; neuroregeneration; oxidative stress; pediatric traumatic brain injury; plasticity; stem cell

Introduction

Traumatic brain injury (TBI) is a major cause of mortality and morbidity in the pediatric population. Children aged 0–4 years old have the highest rate of emergency department (ED) visits. As TBI mortality rates have declined, more children and adolescents are living with TBI-related cognitive and emotional impairments (Cheng et al., 2020). Especially, children who have sustained a brain injury at a younger age are at a high risk of experiencing deficits in cognitive, social, and behavioral sequelae over time, which negatively affects the quality of their life (Neumane et al., 2020). To date there is no therapy that shows a significant benefit in pediatric clinical trials for TBI, and there are unmet needs to develop new age-appropriate treatments for pediatric patients. In this review, we will discuss the uniqueness of pediatric TBI, adult hippocampal neurogenesis after pediatric TBI, factors and potential therapeutics that augment endogenous hippocampal neurogenesis.

Search Strategy and Selection Criteria

This review was compiled by using “PubMed” and “Web of Science Core Collection” within the last 5 years, with an emphasis on the most recent, novel, and comprehensive papers. If the topic did not have relevant information within the last 5 years, we used the most recent paper. Due to the strict limit of 50–100 references, we could not cite all of the relevant publications.

Unique Considerations in the Pathophysiology of Pediatric Traumatic Brain Injury

The pathophysiology of TBI consists of primary and secondary injuries. The primary brain injury is inevitable; however, the secondary brain injury provides a window of treatment. The secondary injury triggers a series of complex pathophysiological events including oxidative stress, neuroinflammation, and apoptosis, leading to disruption of the neural networks and compromise of behavioral and cognitive functions (Ryan et al., 2019). Pediatric TBI populations are heterogenic due to the unique characteristics of the immature brain, including axonal outgrowth, synaptogenesis, and myelination, which exhibit a specific pathological response to brain injury (Sta Maria et al., 2019). Moreover, the heterogeneous outcomes in pediatric TBI patients are determined by mediating variables, such as age at the time of injury. The age at the time of injury is a crucial factor that impacts neuropsychological outcomes. There are neuroanatomical and functional changes at different maturational stages (infant, toddler, and adolescent). Studies in humans show 0–2 years of age is characterized by rapid and dynamic brain development to establish cognitive abilities and behaviors, while brain development after 2 years of age mainly focuses on “fine-tuning” of the existing major circuits and networks (Gilmore et al., 2018). For example, white matter tracks are largely formed at birth, and myelination and

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maturation of existing white matter network develop rapidly after birth (Gilmore et al., 2018). Brain development during adolescence shows region-specific structural and functional changes that correlate with the development of higher cognitive and executive functions. For example, myelination progresses further in the prefrontal cortex, and the thickness and density of the gray matter increase in the primary language cortex (Vijayakumar et al., 2016). Therefore, injuries occurred at different stages of brain development in children can have different impacts on their academic performance and executive functions, some of which only become apparent months or even years after the initial injury.

It has been found that injuries sustained at a younger age can cause more persistent deficits when compared to older children with injuries of similar severity, suggesting that immature brains exhibit increased vulnerability to environmental disruption and heightened susceptibility to insult (Sta Maria et al., 2019). It is well-known that a critical period in brain development correlates with enhanced plasticity and sensitivity which is heavily influenced by environmental demands. Therefore, injury-induced plasticity in an immature brain can alter or even cease brain developmental processes entirely (Hagberg et al., 2015).

In the context of brain injury associated disruption to programmed developmental processes, the immature brain may be more ‘vulnerable’ due to derailed developmental processes and depleted neural resources (Zamani et al., 2020). Infancy and childhood are critical developmental stages associated with rapid dendritic growth and synaptogenesis. Brain insults at infancy and childhood disrupt normal development in the damaged brain regions, alter neural circuitry and cellular environment, and result in delayed recovery in pediatric patients, compared with adult patients with TBI (Zamani et al., 2020). For example, pediatric TBI patients have demonstrated prominent gray and white matter volume loss, compared to age matched controls (Cox et al., 2019). Therefore, the interruption of brain development during critical period places a child at additional risk for neuropsychological deficits beyond those that are the direct result of brain injury. Currently, there is no effective therapy in the treatment of pediatric TBI, and there are urgent needs to develop age-appropriate therapeutics for pediatric patients.

**Dysregulation of Adult Hippocampal Neurogenesis after Pediatric Traumatic Brain Injury**

Evidence shows that recovery from TBI can be limited by the irreversible loss of neurons, leading to significant impairment of cognitive, motor, and emotional functions (Neumane et al., 2020). There is an ever-expanding interest in incorporating adult-born granule cells to the hippocampal circuitry to rescue neuronal loss and cognitive deficits. Stem cell-based therapies and/or cellular therapies have been used to promote tissue replacement, and modify neuroinflammation and immune responses. For example, administration of secretome from umbilical cord mesenchymal stem cells can promote adult hippocampal neurogenesis and improve cognitive function after TBI (Liu et al., 2020). However, the potential therapeutic efficacy is significantly affected by the timing and route of cell delivery, and the cellular microenvironment at the site of the injury (Cox et al., 2019). It has been indicated that neural stem cell (NSC) transplantation in a hostile environment can lead to severe gliosis (Cox et al., 2019). Therefore, stimulation of innate neuro-regenerative mechanisms to support or replace damaged neurons may provide a promising alternative.

Dentate gyrus (DG), a hippocampal subfield, plays an important role in learning and memory. DG is particularly vulnerable to TBI even when it is not directly injured (Zhang et al., 2015a, 2020). Injury-induced neuronal loss in the hippocampal DG has been associated with cognitive deficits after pediatric TBI (Zhang et al., 2020). The DG of hippocampus has the ability for self-renewal through the process of adult neurogenesis in the mammalian brain, including human (Moreno-Jimenez et al., 2019). The adult-born DG granule cells play an important role in learning and memory (Goncalves et al., 2016; Anacker and Hen, 2017; Miller and Sahay, 2019), and emotional regulation (Yun et al., 2016). Although adult neurogenesis occurs throughout life, it decreases with age in adult humans (Sorrells et al., 2018). The immature adult hippocampal neurogenesis has been correlated with cognitive decline during aging and in neurodegenerative disorders (Moreno-Jimenez et al., 2019).

The outcomes of pediatric TBI are impacted by the severity of injury, which is typically categorized as mild, moderate, or severe. More severe injuries, such as larger, more diffused, and bilateral injuries, are associated with worse physical and cognitive performance in pediatric patients with TBI (Neumane et al., 2020). Studies have shown an enhanced NSC proliferation in correspondence to increased TBI severity, leading to compensation of mature neuron loss (Wang et al., 2016). Longitudinal studies indicate that mild to moderate TBI recovery typically shows an asymptotic pattern in human patients, with rapid improvement within the first weeks and months of injury, followed by a slower rate of improvement, and a plateau or even deterioration afterwards (Ledoux et al., 2019). Interestingly, TBI can induce an acute upregulation of newborn neurons, followed by a chronic reduction of baseline neurogenesis, which may be due to the depletion of neural progenitors and/or the death of immature hippocampal neurons (Ngwenya and Danzer, 2018). Therefore, the neuronal loss and impaired endogenous neurogenesis in the hippocampus may contribute to TBI-induced neuropathological outcomes.

In theory, augmentation of adult hippocampal neurogenesis after TBI could facilitate cognitive recovery (Villasana et al., 2015). However, neurons born after various neuronal injuries can have morphological and functional abnormalities, which can result in negative outcomes (Ibrahim et al., 2016). For example, TBI results in aberrant migration and impaired dendrite development of adult-born neurons, which may cause dysregulated neural network connectivity (Ibrahim et al., 2016; Zhang et al., 2020). Therefore, a better understanding of post-injury hippocampal neurogenesis can facilitate a more successful repair of the damaged immature brain.

**Factors that Affect Adult Hippocampal Neurogenesis**

Adult hippocampal neurogenesis is a complex process, which includes NSC proliferation, differentiation, migration, maturation, and functional integration into the existing neuronal network (Alvarez et al., 2016). Adult hippocampal neurogenesis is dynamically regulated by a number of intrinsic as well as extrinsic factors (Vicidomini et al., 2020). A variety of therapeutic approaches have been proposed for promoting post-injury neurogenesis and ameliorating secondary injuries after TBI. Manipulations, such as exercise, and an enriched environment increase adult hippocampal neurogenesis (Alvarez et al., 2016). However, a successful regenerative response to injury requires not only NSC proliferation but also proper migration and integration of new neurons into the existing hippocampal circuitry (Villasana et al., 2015; Ibrahim et al., 2016). aberrant migration of adult-born neurons can lead to the malfunction of neural network and increase seizure susceptibility (Villasana et al., 2015). Developing effective therapeutics for neuroprotection or neurorestoration is particularly difficult due to the complexity and heterogeneity of TBI. Dynamic interactions between inflammatory and
metabolic pathways is a hallmark of secondary injury after TBI, therefore, a successful therapy requires targeting multiple injury pathways. In the following section, we will discuss the impact of neuroinflammation, mitochondrial oxidative stress, lipid metabolism, tryptophan metabolism, and epigenetic regulation on the adult hippocampal neurogenesis after pediatric TBI (Figure 1).

The role of neuroinflammation

Neuroinflammation, a secondary injury response following TBI, plays an important role in determining TBI outcomes. Neuroinflammation is a complex interaction between neurons and glial cells (e.g., astrocytes and microglia), and soluble components (e.g., cytokines and chemokines), which can be beneficial for debris clearance, and regulation of injured tissues. However, dysregulated neuroinflammation can cause more neuronal death and progressive neurodegeneration. The neurogenic niche, an intrinsic microenvironment, regulates adult-born neurons by different components (Sofroniew, 2015; Vicidomini et al., 2020). Neuroinflammation at the neurogenic niche is regulated, in part, by activated microglia, the resident macrophages of the brain, which plays an important role in maintaining central nervous system homeostasis under physiological conditions (Vicidomini et al., 2020). Microglia are highly proliferative, and regulate synaptic pruning and survival of newborn neurons (Weinhard et al., 2018). Moreover, the neuron-microglia interaction determines the distinct profiles of microglia-secreted chemokines and cytokines. For example, neuron-derived fractalkine (CX3CL1; FKN) acting on the CX3CR1 receptors expressed in microglia can promote adult hippocampal neurogenesis (Vicidomini et al., 2020). TBI-induced microglial activation can be both detrimental and beneficial. For example, microglial activation can increase nitric oxide synthesis and pro-inflammatory cytokine release, which leads to blood-brain barrier (BBB) dysfunction and neurodegeneration (Corrigan et al., 2016; Kumar et al., 2017). However, microglia can also serve a neuroprotective role by clearing damaged cell debris, releasing anti-inflammatory cytokines, and promoting tissue repair (Russo and McGavern, 2016; Willis et al., 2020).

Neuroinflammation and long-lasting microglial activation play a key role in the cognitive deficits and impairment of adult hippocampal neurogenesis following pediatric TBI (Zhang et al., 2015a, 2020). Microglia are involved in all of the steps of adult hippocampal neurogenesis, including NSC proliferation, differentiation, and incorporation into the existing neural network. Microglial activation can be correlated with increased or decreased neurogenesis, which may be due to the heterogeneity of microglia (Masuda et al., 2019). Pro-inflammatory microglia release pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, which increase the death of neurogenic NSC lineage, and decrease the complexity of neurites of immature newborn neurons (Hueston et al., 2018). However, the role of microglia after TBI is still contradictory. A recent study shows that the removal of microglia has little effect on the outcome of TBI, but induction of a neuroprotective microglial phenotype can profoundly improve the survival of newborn neurons and alleviate cognitive function via IL-6/IL-6 receptor (IL-6R) signaling pathway (Willis et al., 2020).

Astrocytes maintain BBB integrity, modify neuronal excitability and metabolism, and regulate synapse formation and neurotransmission (Farhy-Tselnicker et al., 2017; Blanco-Suarez et al., 2018). Astrocytes provide structural and functional support for NSC proliferation, differentiation and maturation in the neurogenic niche (Vicidomini et al., 2020). Astrocytes express a variety of membrane-bound and secreted factors that regulate the development and maturation of newborn neurons (Sultan et al., 2015; Clarke et al., 2018). Astrocytes undergo morphological changes after TBI, and secrete both pro-inflammatory cytokines such as IL-1β, and neurotrophic factors such as transforming growth factor beta (TGF-β) (Clement et al., 2020). Therefore, reactive astrocytes act as a double-edged sword, and plays an important role in pathophysiological processes after pediatric TBI (Clement et al., 2020). Excessive activation of astrocytes potentiate inflammatory response and lead to formation of glial scars, which limits neural regeneration and neuroplasticity.

Oligodendrocyte progenitor cells (OPCs), a subtype of glial cells in the central nervous system, provide the basis for myelination in their mature form as oligodendrocytes (OLGs). TBI can cause traumatic axonal injuries (TAI), leading to demyelination in intact axons and oligodendrocyte death. Axon recovery after TBI becomes extremely dependent on the capabilities of OPCs and their contribution to myelin regeneration. Evidence indicates that neuroinflammation dynamically regulates the proliferation and recruitment of OPCs following TBI. For example, pro-inflammatory cytokines can initiate the death of proliferative OPCs and mature oligodendrocytes (Braun et al., 2017), which might be responsible for progressive white matter injury and long-term functional deficits in pediatric TBI patients (Dennis et al., 2015; Genc et al., 2017).

Neuroinflammation is a complex process that can enhance or suppress neurogenesis, depending on the phenotypes of glia cells, and the duration of inflammation. Therefore, a better understanding of the entire neuroinflammatory cascade can facilitate targeted anti-inflammatory treatments to improve outcomes following TBI.

The role of lipid metabolism

The highly variable outcomes after pediatric TBI can be attributed to the intrinsic nature of the developing brain, including lipid metabolism. Lipids are essential for a variety of functions, including myelination, membrane integrity, and neurotransmission (Bowman et al., 2019). Polyunsaturated fatty acids, such as arachidonic acid and docosahexaenoic acid (DHA), are particularly abundant in brain phospholipids and essential for axonal outgrowth. Polysaturated fatty acids are a target for lipid peroxidation following TBI due to their highly oxidizable structure, generating numerous oxidized free fatty acid (FFA) that correlate with both injury severity and mortality in human patients with TBI (Bowman et al., 2019). Oxidized FFA are important signaling molecules involved in numerous cellular responses, including neuroinflammation (Dennis and Norris, 2015). Studies demonstrate that FFA metabolism significantly changed after pediatric TBI (Chitturi et al., 2018). Increased FFA following TBI is associated with poor outcomes by promoting apoptosis and stimulating pro-inflammatory responses (Chen et al., 2017). Oxidized FFA can be produced...
via calcium-dependent or mitochondrial-based/calcium-independent pathways (Yurina et al., 2014). In the calcium-dependent pathway, the polyunsaturated fatty acids derived from cellular and organellar membranes are hydrolyzed from phospholipid precursors via calcium-dependent phospholipase A2 (PLA2), and subsequently peroxidized by cyclooxygenases, lipoxygenases, and cytochrome P450 (Yurina et al., 2014). Cytochrome P450 enzymes are monooxygenase and oxidize arachidonic acid to eicosydocosatrienoic acids and hydroxy-eicosatetraenoic acids. The cytochrome P450 4A (CYP4A) catalyzes the ω-hydroxylation of arachidonic acid to 20-hydroxyicosatetraenoic acid (20-HETE) (Yurina et al., 2014). Studies have shown that this can increase reactive oxygen species and promote inflammation. Administration of a 20-HEHE synthesis inhibitor improves outcome after pediatric TBI (Shu et al., 2019).

Lipid metabolism can also influence proliferation and differentiation of adult neural progenitors (Knobloch et al., 2017). Oxidized fatty acids play an important role in energy production, and inhibition of fatty acid oxidation in hippocampal neurogenesis leads to increased quiescent NSC death and reduced NSC proliferation (Knobloch et al., 2017). Evidence shows defects in oxidized fatty acids lead to enhanced progenitor generation, but subsequently reduce embryonic NSC pool during brain development (Xie et al., 2016). Aberrant lipid metabolism in the neurogenic niche reduces NPC differentiation towards the neuronal lineage (Engel et al., 2019).

Lipid metabolism is regulated by different factors, such as apolipoprotein E (ApoE) and adiponectin. ApoE is mainly secreted by astrocytes and regulates lipid transport and homeostasis, and supports neuronal development, beta-amyloid metabolism, and BBB integrity (Koizumi et al., 2018). There are three major isoforms of ApoE, including ApoE2, ApoE3, and ApoE4. ApoE4 is associated with late-onset Alzheimer’s disease, cognitive impairment, and tau hyper-phosphorylation after TBI (Koizumi et al., 2018). Injury can induce rapid ApoE synthesis in neurons, which participate in lipid transport and redistribution for membrane repair and remodeling. Studies have shown that ApoE mimetic peptide reduces BBB disruption, tau accumulation, inflammatory microglia activation, and ameliorates brain edema and neuronal degeneration (Qin et al., 2017). However, neurons can generate abnormal and neurotoxic ApoE fragments, especially ApoE4, which can be targeted for proteolytic cleavage, and translocate into the cytosol, leading to mitochondrial dysfunction and neurodegeneration (Mahley and Huang, 2012). ApoE4-associated phospholipid dysregulation impairs BBB integrity and results in tau hyperphosphorylation after TBI (Cao et al., 2017; Teng et al., 2017). Studies indicate that ApoE regulates the development of adult newborn hippocampal neurons, and modulates injury-induced dendritogenesis and synaptogenesis of hippocampal NSCs (Tensaouti et al., 2020). Moreover, ApoE acts as a negative regulator of NSC proliferation at later ages of injury when the progenitor pool is depleted (Tensaouti et al., 2020), while ApoE ablation shifts NSPC differentiation towards astrogenesis instead of neurogenesis (Hong et al., 2016).

Adiponectin is involved in several physiological processes, such as glucose and lipid homeostasis, neuroinflammation, and neurogenesis (Bloemer et al., 2018). Two major types of adiponectin receptors, AdipoR1 and AdipoR2, are expressed in different brain regions, including the neurogenic niche at hippocampal DG (Bloemer et al., 2018). Adiponectin increases proliferation of hippocampal NSCs, mediates physical exercise-induced hippocampal neurogenesis, and reduces depression-like behaviors (Yau et al., 2015). Adiponectin deficiency reduces NSC proliferation and differentiation, decreases dendritic length and complexity of DG granule cells, and increases susceptibility to cognitive deficits and depression (Zhang et al., 2016a, 2017).

Abnormal lipid metabolism contributes to the secondary injury following pediatric TBI. The transient metabolic disturbance after pediatric TBI can interrupt highly orchestrated metabolic processes that are essential for health impairing development, leading to long-term impairment of NSC function. Evidence indicates that the increase in neurogenic response at the acute phase after TBI is transient, while the long-term survival rate of newly formed neurons is reduced compared to control levels (Hong et al., 2016). Therefore, therapeutic strategies that can reduce delayed and progressive neurodegeneration is an area that needs to be emphasized in future research.

The role of mitochondria and oxidative stress
Mitochondria play an important role in ATP synthesis, intracellular calcium buffering, oxidative stress, and apoptosis (Fischer et al., 2016). Under physiological conditions, mitochondria undergo fission and fusion to maintain metabolic homeostasis; however, TBI can induce an imbalance in this process, which contributes to neuronal death (Balog et al., 2016). For example, abnormally increased fission can cause mitochondria fragmentation, while inhibition of fission can reduce the loss of hippocampal neurons and improve learning and memory after TBI (Wu et al., 2016). Mitochondria also play a key role in the proliferative and differentiation of NSCs. Mitochondrial inhibition promotes selective death of immature adult-born neurons, while mitochondrial protection improves survival of immature adult-born neurons under inflammatory conditions (Voloboueva et al., 2017).

The synthesis of reactive oxygen species and reactive nitrogen species, such as nitrous oxide increases after TBI, which is proportional to lipid peroxidation, and causes DNA damage (Kumar Sahel et al., 2019). This is signified by the decrease in antioxidants such as superoxide dismutase, glutathione, and glutathione peroxidase. The alteration in the pro- and antioxidant balance results in oxidative stress, which is linked to axonal injury, impaired synaptic plasticity, and cognitive deficits (Corrigan et al., 2016). Oxidative stress induces acute structural and functional damage to mitochondria, impairs ATP synthesis, and contributes to cell death and poor cognitive outcomes (Fischer et al., 2016). The damages to the mitochondria, such as calcium overload, can result in energy failure, and increase reactive oxygen species production, which further enhances oxidative stress (Kumar Sahel et al., 2019). Drugs that target mitochondrial dysfunction, specifically in the diseased cells, can offer neuroprotection and improve cognition following TBI.

The role of tryptophan metabolism
Pro-inflammatory cytokines, such as IL-1β, can affect neurogenesis via alterations of the tryptophan (TRP)-kynurenine (KYN) pathway (Borsini et al., 2017). TRP, an essential amino acid, is required for protein synthesis to ensure cell survival, and plays an important role in health and disease (Comai et al., 2020). TRP can be metabolized into KYN by indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). Under physiological conditions, KYN is mainly metabolized via a neuroprotective pathway into kynurenic acid (KYN), a N-methyl-D-aspartate (NMDA) receptor antagonist, by kynurenine-amino-transferase expressed by astrocytes. Under pathological conditions, such as neuroinflammation, KYN is catabolized via a “neurotoxic pathway” into quinolinic acid (QUIN), a NMDA receptor agonist and co-enzyme of nicotinamide adenine dinucleotide (NAD+) by kynurenine monoxygenase (KMO) expressed in microglia (Comai et al., 2020). Therefore, QUIN/KYN ratio, an indicator of the homeostasis of glutamatergic neurotransmission, is dynamically regulated by microglia.
TRP can also be hydroxylated to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH), and decarboxylated into 5-hydroxytryptamine (5-HT; serotonin) by aromatic amino acid decarboxylase (DDC). 5-HT is further metabolized to 5-HIAA via monoamine oxidase (MAO-A) and aldehyde dehydrogenase (AD). Meanwhile, 5-HT can also be converted into N-acetylseryosoritin via serotonin-N-acetyltransferase, and is then transformed to melatonin (MLT) by hydroxyindole-O-methyltransferase. 5-HT and MLT are implicated in regulating many processes including mood, sleep, and inflammation (Comai et al., 2020). Studies show that melatonin modulates NSC differentiation in the DG, and enhances cell survival and dendrite maturation of new-born neurons (Figueiro-Silva et al., 2018). Pediatric TBI switches TRP catabolism from serotonin pathway to the KYN pathway, and leads to melatonin depletion (Zhang et al., 2018). The melatonin depletion might contribute to the impaired hippocampal neurogenesis and cognitive deficits in pediatric TBI (Zhang et al., 2020).

The role of epigenetic regulation
The density and function of NSCs in the developing brain are not only regulated by extrinsic factors in the neurogenic niche, but also modulated by intrinsic molecular mechanisms. Epigenetic mechanisms, such as DNA methylation, histone modification, as well as pre- and post-transcriptional modifications, can control proliferation, differentiation and maturation of NSCs in the DG. For example, DNA methylation regulates Notch signaling target gene activity and differentially modulates the proliferation of NSCs after TBI (Zhang et al., 2015b). Histone deacetylation 4 downregulation after TBI induces aberrant activation of the Pax3-Ngn2 signaling pathway, leading to the impairment of hippocampal neurogenesis (Saha et al., 2019). To date, few studies have explored the role of epigenetic regulations of hippocampal NSCs after pediatric TBI, especially between acute injury and long-term impact on NSC’s function and pool size. Understanding the molecular mechanisms involved in the epigenetic regulation of NSCs will aid in advancing our knowledge of the role of hippocampal neurogenesis in degeneration and regeneration after TBI. These epigenetic mechanisms can facilitate the development of therapeutics that promote long-lasting self-renewal of NSCs.

Potential Therapeutics Targeting Adult Hippocampal Neurogenesis after Pediatric Traumatic Brain Injury
Neuroprotective strategies targeting secondary injuries after TBI have been explored in both pre-clinical animal models and clinical trials. We will discuss pharmaceutical agents that have been approved by U.S. Food and Drug Administration (FDA) and currently used in clinical trials.

N-acetylcyesteine
N-acetylcyesteine (NAC) is a US FDA-approved pharmacological agent that shows neuroprotective agent effects in central and peripheral nervous system injuries (Hoffer et al., 2017). N-acetylcyesteine amide (NACA), an amide derivate of NAC, has improved hydrophobicity and lipophilicity and a prolonged plasmatic half-life, leading to increased penetration into the BBB, mitochondria, and other cellular constituents (Bhatti et al., 2017). NAC and NACA have antioxidant and anti-inflammatory properties, which increase brain glutathione, and decrease neuroinflammation and oxidative stress in preclinical animal models of TBI (Hoffer et al., 2017). Clinical studies have shown that NAC decreased neuroinflammation and oxidative stress, reduced imbalance and headache following TBI in military personnel, and improved cognitive functioning and brain perfusion in retired professional football players (Bhatti et al., 2017). Studies using a combination therapy have demonstrated a greater potency using synergistic drug combinations. For example, the combination of minocycline and NAC synergistically prevented cognitive deficits in a mouse model of TBI (Sangobowale et al., 2018). However, the therapeutic efficacy of NAC in pediatric patients remain inconclusive. For example, a recent phase I randomized clinical trial conducted in pediatric patients (2–18 years of age) demonstrates that co-administration of NAC and probenecid (an antimicrobial agent) increases bioavailability of NAC and probenecid without adverse effects. However, this combination therapy did not improve the Glasgow outcome scale upon hospital discharge or at 3 months follow-up (Clark et al., 2017). Although studies conducted in animal models can provide insight and guidance to NAC use in TBI patients, the translation strategies require major efforts and collaboration between clinicians and scientists.

Minocycline
Minocycline is a well characterized, safe, and FDA approved anti-inflammatory drug, and has been used experimentally and clinically. Minocycline has high BBB permeability, decreases neuroinflammation, prevents injury-induced hyperthermia, and improves neurological function in a preclinical model of TBI (Taylor et al., 2018). In addition, minocycline treatment reduces high-mobility group box protein 1 (HMGB1) translocation to cytoplasm, attenuates microglial activation, and improves cognition after pediatric TBI (Simon et al., 2018). Although minocycline reduces neuroinflammation and improves neurological outcome, it does not increase neurogenesis (Ng et al., 2012). Clinical studies of minocycline in TBI have reported varying results. In a recent study, minocycline was administered to patients with moderate to severe TBI at a dose twice that as recommended for treatment of infection, leading to an improved outcome (Meythaler et al., 2019). However, another study indicates that minocycline treatment reduced chronic microglial activation, but increased brain atrophy and neurodegeneration (Scott et al., 2018).

Docosahexaenoic acid
Studies suggest that FFA contributes to the secondary injury after TBI. Enhancing mitochondrial function and FFA oxidation in mitochondria after TBI may improve neuroprotection to lessen post-injury cell death (Bowman et al., 2019). Omega-3 polyunsaturated fatty acid (ω-3 PUFAs), such as DHA, provides energy support through lipid synthesis, regulates inflammatory response, and exhibits antioxidative and anti-inflammatory effects by attenuating proinflammatory microglial activation (Chen et al., 2017). Endogenous synthesis of DHA from alpha-linoleic acid (ALA) is very limited in mammals. Therefore, DHA accrual depends mainly upon dietary intake (Zarate et al., 2017). Under physiological conditions, DHA is a critical "building block" that esterified into membrane phospholipids (Zarate et al., 2017). Under pathological conditions, such as TBI, ATP level decreases after injury, which prevents the reutilization of DHA via re-esterification, and results in the loss of DHA released from disrupted neural membranes. DHA deficiency is associated with poor outcomes after experimental TBI (Desai et al., 2016). Pediatric TBI decreases brain DHA content, and DHA treatment ameliorates oxidative stress, reducing oxidative brain injury, and improves neurological outcome (Simon et al., 2018).
stress, inflammation, white matter injury, and improves neurologic outcomes in pediatric preclinical TBI models (Schober et al., 2020). However, these effects have been less conclusive in humans due to the heterogeneity of injury, and the differences in endogenous DHA synthesis and recycling in human patients.

**Melatonin**

Melatonin is a promising, well-tolerated, neuroprotective agent, and is recommended as part of the management plan of pediatric TBI (Santini et al., 2018). Melatonin reduces oxidative stress, neuroinflammation, and neurodegeneration after TBI (Barlow et al., 2019; Rehman et al., 2019). Amelioration of oxidative stress can create a favorable milieu for NSCs and can be an effective therapeutic approach for neuroprotection and neurofacilitation following TBI. Melatonin activates inhibitory gamma-aminobutyric acid (GABA) receptors, especially GABA_A receptors, which counter-balance glutamate excitotoxicity (Barlow et al., 2019). Melatonin offers therapeutic potential for many of the common post-TBI symptoms such as sleep disruption and mood disturbance (Barlow et al., 2019). A recent study has demonstrated that N-acetyl serotonin, a precursor of melatonin, has strong antioxidant and anti-apoptotic effects by activation of tropomyosin-related kinase receptor B-mediated signaling cascades. N-acetyl serotonin improves hippocampal neurogenesis and ameliorates cognitive impairments after TBI (Li et al., 2019). Although melatonin significantly improved neurological, cognitive, and motor function in pre-clinical animal models (Barlow et al., 2019), the efficacy of melatonin remains controversial in the treatment of pediatric TBI patients. In a randomized, double-blinded study, melatonin decreased hyperactivity in pediatric patients, but did not improve the overall outcomes (Barlow et al., 2020).

**Nanoparticles-guided drug delivery**

Most drugs undergoing preclinical and clinical trials for TBI lack the ability to target specific tissues, cells and organelles. Nanoparticles, such as dendrimers, may be helpful by delivering therapeutics specifically to target cells and organelles (Nance et al., 2016; Zhang et al., 2016b). For example, dendrimer-conjugated NAC, dendrimer-conjugated minocycline, and dendrimer-conjugated sinonemine have shown significant promise in target delivery of NAC, minocycline, and sinonemine to activated microglia and astrocytes at the site of brain injury, producing remarkable improvements in neurological outcomes at a lower dose than non-conjugated free drugs (Nance et al., 2017; Sharma et al., 2017, 2018b, 2020). In pre-clinical animal models of pediatric TBI, dendrimer-mediated delivery can target microglial mitochondria at the site of brain injury, which provides a useful tool for reducing mitochondrial dysfunction and oxidative stress (Sharma et al., 2018a). Considering the crucial role that the microglia plays in NSC development and maturation, designing therapies that target microglia cells at the appropriate time points may facilitate long-term recovery after pediatric TBI.

**Therapeutic time window and long-term outcomes**

The translation of positive pre-clinical findings targeting acute neuroprotection fails to improve long-term functional outcomes in clinical trials of TBI (Bramlett and Dietrich, 2015). Therapeutic time window plays a critical role in the treatment of TBI because most drugs lose efficacy with increasing intervals between the onset of injury and the time to first treatment (Mohamadpour et al., 2019). The therapeutics mentioned above are mostly utilized in the acute phase of injury, and the therapeutic efficacy at different time windows post-injury were not analyzed. In addition, multiple outcome measures, such as cognition, motor, and psychosocial outcomes should be performed during the chronic phase of injury following a therapeutic intervention. It is anticipated that combinational approaches that target different cellular and molecular pathways can extend the therapeutic time window in the treatment of chronic consequences of TBI, and ultimately improve the quality of life in pediatric patients.

**Concluding Remarks and Future Directions**

In this review, we have taken a holistic view of hippocampal neurogenesis after pediatric TBI, beginning with characteristics of pediatric TBI leading to hippocampal neurogenesis impairment and progressing to potential therapeutics for promoting endogenous neurogenesis and regeneration. We highlight neuroinflammation, lipid metabolism, mitochondrial dysfunction, oxidative stress, tryptophan metabolism, and epigenetic regulation as promising avenues for developing novel therapeutics (Figures 1 and 2).

As detailed above, it is well established that secondary injuries post-TBI constitute complex and dynamic responses. Several pharmacological neuroprotective agents have shown promise in pre-clinical models and early phase II clinical trials, but failed to show positive outcomes in larger, phase III trials. This indicates that a multi-pronged approach that targets several pathways may be necessary for enhancing endogenous neurogenesis and improving plasticity and repair. Future pre-clinical studies and clinical trials with multi-mechanistic combinational neuroprotective approaches are urgently needed; however, these studies need to be meticulously designed. Variations in the dosage and duration of the therapeutic agents have to be carefully considered when being tested in pediatric patients since there are differences between the mature and the developing brains.

In conclusion, the discoveries reported in this review may pave the way for future therapeutic interventions that involve enhancing adult hippocampal neurogenesis, promoting the functional incorporation of new neurons into affected neural circuits, and facilitating repair and restoration of brain functions in pediatric TBI patients.
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