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Advanced nanotherapies to promote neuroregeneration in the injured newborn brain

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ABSTRACT

Neonatal brain injury affects thousands of babies each year and may lead to long-term and permanent physical and neurological problems. Currently, therapeutic hypothermia is standard clinical care for term newborns with moderate to severe neonatal encephalopathy. Nevertheless, it is not completely protective, and additional strategies to restore and promote regeneration are urgently needed. One way to ensure recovery following injury to the immature brain is to augment endogenous regenerative pathways. However, novel strategies such as stem cell therapy, gene therapies and nanotechnology have not been adequately explored in this unique age group. In this perspective review, we describe current efforts that promote neuroprotection and potential targets that are unique to the developing brain, which can be leveraged to facilitate neuroregeneration.

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1. Introduction

Neonatal brain injury affects millions of babies each year and often leads to long-term and permanent physical and neurological problems such as mental retardation, seizures, visual and hearing impairment, learning and behavioral disabilities, attention deficits and hyperactivity [1–5]. Brain injury in the term infant is clinically recognized by a unique encephalopathy characterized by difficulty in initiating and maintaining respiration, depression of tone and reflexes, and altered level of consciousness [6]. The presence of neonatal encephalopathy (NE) is ominous since 15-20% of affected babies die in the newborn period and an additional 25% develop permanent neurological damage [7,8]. NE occurs in 6-8 per 1000 live births in high-income countries whereas this incidence increases up to 20 per 1000 live births in low and middle-income countries [9]. In 2010, 1.15 million babies were calculated to have developed NE associated with intrapartum events, with 96% born in low- and middle-income countries [4]. The causes of neonatal brain injury and NE are multiple and often multifactorial. Perinatal asphyxia, defined as oxygen deprivation that occurs around the time of birth, can cause NE if it is severe and long enough [10], and it is one of the principal causes of early neonatal death or disability in survivors, in spite of all the improvements in perinatal intensive care medicine [11,12]. Diseases such as perinatal stroke and asphyxia leading to brain injury share common characteristics in their progression, eventually resulting in neuronal death. Such impairment almost always renders the brain incapable of fully regenerating and eventually leads to death or long-term and permanent physical and neurological problems for these infants.

A greater understanding of these mechanisms would provide opportunities to intervene therapeutically and to avoid unwanted off target effects in newborns [7,13]. Currently, newborns with moderate to severe NE receive therapeutic hypothermia (TH) [14] that involves cooling the body or brain for the purpose of preserving the organs, particularly improving brain viability [15]. This treatment is now established as a standard clinical care for term newborns with moderate to severe NE in high-income countries [16–18]. With this protocol of cooling the brain for 72h started within the first 6 hours of life, brain damage is partially diminished [19–22]. Experimental evidence suggests that newborns should be treated with mild hypothermia as early as possible before secondary energy failure starts. Additionally, TH should last at least until the resolution of secondary energy failure to ensure a potent and long-lasting neuroprotection, since deleterious inflammation may be reactivated by premature rewarming [6,23].

In fact, TH is able to reduce adverse outcomes from 60 to 45% based on results from 11 randomized controlled human trials involving 1505 term and late preterm neonates with moderate or severe encephalopathy and evidence of intrapartum asphyxia. These studies concluded that TH significantly reduced the mortality rate or major neurodevelopmental disability [19–22]. Although the mechanisms of action of TH are still unclear, there is evidence from experimental data that protective mechanisms are multifactorial. TH is able to reduce cerebral metabolism, decrease free radical formation such as nitric oxide and superoxide, inhibit the pro-inflammatory reaction and reduce accumulation of excitatory amino acids, besides suppressing many of the pathways leading to delayed and programmed cell death [17,23]. Nevertheless, almost 40% of treated infants have adverse outcomes [19–22]. In addition, this therapy does not address the preterm population, or babies suffering from stroke, infection or trauma [6] and it may not be feasible in low- and middle-income countries [4]. Thus, adjunctive strategies are urgently needed to improve the outcome for these neonates.

One way of enabling recovery after perinatal brain injury would be to supplement the naturally occurring endogenous pathways to recover the brain, such as neurogenesis and oligodendrogenesis. Neurogenesis in the developing human brain is a physiological process, but the production of new neurons in adulthood has been deeply debated. The initial discovery by Altman and Gopal, that neurogenic niches exist in the subventricular zone (SVZ) [24] and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) of the adult mammalian brain [25] led to the questioning of the prevailing hypothesis that the brain is a post-mitotic structure, incapable of generating new neurons. Ever since, numerous studies have verified that neurogenesis is present in the postnatal brain [26] and carries on, although in a declining trend, throughout adulthood [27]. Understanding better how healthy brains change over time could give us a clue about how to enhance the generation of new neurons. Hence, it is reasonable to assume that preserving or potentiating the production of new neurons can be beneficial for the motor and cognitive decline observed in children and adults with neurodegeneration, and research is underway for ways to aid the brain in orchestrating a self-repair response to compensate for the acute or chronic neuronal loss. Similarly, promoting oligodendrogenesis is also crucial in the recovery process after neonatal brain injury. Immature oligodendrocytes are highly vulnerable to HI injury [28] and impairments in white matter development due to neonatal brain injury usually lead to disruption of myelination [29,30]. Thus, oligodendrocyte regeneration and enhanced myelination would prevent future neurodevelopmental deficits.

Although there has been an increase in developing innovative therapeutics for targeting neurodegenerative disorders in adulthood, such strategies have not been explored adequately in the neonatal and pediatric population. The bulk of cognitive and motor development occurs in the first few years of life with a significant increase in brain volume by ~101% in the first year of life [31]. An injury during this vulnerable time period will affect normal physiologic neurogenesis and growth. In spite of the large social, economic and emotional burden of neonatal brain injury, there has not been a significant advancement in the number of clinical stage therapies that have been developed for this patient population. However, there is also greater potential for novel therapies to have an impact in this population, since they can be targeted to restoring the endogenous and physiologic regenerative pathways that are inherently increased at this age. In this review, we will first provide an overview of the pathophysiological pathways involved in neonatal brain injury and key factors that encourage neural healing. Subsequently, we will discuss recent advances in the various gene, cell and nanotechnology based therapies with relevance to its application in neonatal brain injury along with potential areas for future research.

2. Etiology and pathophysiology of neonatal brain injury

2.1. Common causes of brain injury in the perinatal period

Brain damage to the baby around the time of birth can be caused by many factors, including hypoxia-ischemia (HI), intraventricular hemorrhage, infection or chorioamnionitis, perinatal stroke, jaundice or physical trauma suffered during labor and delivery [7,32,33]. Genetic factors such as copy number variates and single gene mutations, epigenetic programming [34] and alterations in the microbiome-gut-brain axis [35,36] have also been implicated in neonatal brain injury. Although perinatal brain injury affects infants born at all gestational ages, the incidence and morbidity increases with decreasing gestational age [37]. The resulting damage to the infant's brain will also vary depending on the duration and severity of the injury, and where and how the injury occurred [38]. While full-term babies sustain selective damage to sensorimotor cortex, basal ganglia, thalamus and brain stem after HI, the periventricular white matter is more susceptible in preterm newborns [11]. Animal studies have shown that there are characteristically vulnerable cell populations that exhibit sensitivity to HI and excitotoxic insults [39]. In the term brain, projection neurons especially in the deep gray nuclei are at greatest risk during HI insults, while in the preterm brain, the subplate neurons and oligodendrocyte precursors are most vulnerable, resulting in greater white matter injury [40].

Periventricular leukomalacia (PVL) or periventricular white matter injury is a consistent and well-described neuropathological correlate in preterm infants. PVL occurs due to inadequate arterial blood supply to deep white matter, which leads to the necrosis of small areas of brain tissue around ventricles and surrounding edema [35,36]. PVL disrupts the normal progression of developmental myelination, since there is loss of late oligodendrocyte progenitors triggered by oxidative stress and other insults [41,42]. Both ischemic and inflammatory mechanisms are involved in the etiology of white matter injury of prematurity [42], as well as hypoxia alone may contribute to white matter abnormalities in the preterm population [43]. In the case of premature babies affected by PVL, they may have no outward signs or symptoms of the disorder, but they are at risk for motor disorders, delayed mental development, coordination problems, and vision and hearing impairments [44]. PVL is known to be an independent risk factor for the development of cerebral palsy, a chronic childhood condition characterized by disorders in the development of movement and posture that may often be accompanied by disturbances of cognition and behavior [45].

Another cause of neonatal brain injury is perinatal stroke which is defined as an acute neurologic syndrome with chronic sequelae due to cerebral injury of vascular origin occurring between 20 weeks gestation and 28 days after birth. Perinatal stroke may occur due to focal cerebral injury due to arterial ischemic stroke, cerebral venous thrombosis, or primary intracerebral hemorrhage. The incidence of perinatal stroke is high, occurring in approximately 1 in 2,000 births, and is similar to the incidence of stroke in the elderly [46]. Moreover, perinatal strokes account for most cases of hemiparetic cerebral palsy [47]. In fact, more than half of all children with cerebral palsy are born at term and in many instances the etiology is related to some form of birth related cerebrovascular focal or global insult [46]. The symptoms associated with a neonatal stroke depend on the size and the cause of the stroke. The most common symptom of neonatal stroke are seizures, and anticonvulsant medications are used to limit or stop seizures, even if they have side effects that can affect recovery after brain injury [46]. Current therapies are focused on alleviation of symptoms and prevention of secondary injury but not on addressing the primary insult.

2.2. Pathophysiological mechanisms in neonatal hypoxic-ischemic brain injury

Although all the above mentioned etiologies of perinatal brain injury are separate clinical entities, they remain interrelated by their risk factors and pathogenesis, which is multifactorial [37,48]. The pathophysiological mechanisms of HI brain injury are divided into three phases (Fig. 1). Even if the cellular targets of HI are different depending on the age and severity of insult, the basic multifaceted cascade of injury occurs uniformly regardless of age and continues for a prolonged period of time [38].

After HI, there is rapid depletion of adenosine triphosphate (ATP) because of the decline in oxidative phosphorylation. Cells are able to switch to anaerobic metabolism, but this rapidly becomes energetically inefficient and results in the collapse of ATP-dependent Na/K pump and accumulation of metabolites, including lactic acid and hypoxanthine. This is followed by progressive membrane depolarization, with a disproportionate accumulation of excitatory amino acids in the extracellular side, as well as an excessive entry of water, sodium and calcium into the cell. As a result of all these primary processes, cellular edema and early cell death ensue [49–51]. After reoxygenation and reperfusion, there is partial recovery of oxidative metabolism, but this leads to an elevated generation of reactive oxygen species (ROS), higher amount of intracellular calcium and mitochondrial dysfunction. Simultaneously, there is an augmentation in the expression of pro-inflammatory genes. These events, occurring during the secondary phase, which is marked by the onset of seizures [52], contribute to late cell death [6,14,50]. Finally, during the tertiary phase, which can last from days to months, harmful inflammation and epigenomic changes occur while neurogenesis, synaptogenesis and axonal growth are diminished, leading to even greater brain injury [53]. In short, there is a primary energy failure due to the insult, followed by a secondary phase, which is a consequence of reoxygenation and reperfusion and, finally, a tertiary phase where there is ongoing injury and inflammation becomes chronic [12,54,55].

2.3. Unique considerations in the pathophysiology of neonatal brain injury

There are substantial differences in the pathophysiological responses and repair mechanisms between neonatal and adult brains following an injury [53]. The neonatal brain differs markedly from the mature nervous brain in its response to HI, and may be more vulnerable than the mature brain due to a greater susceptibility to oxidative stress [56]. The immature brain is uniquely and exquisitely sensitive to oxidative stress, which accounts for the cell death seen after perinatal asphyxia, because of its high concentration of unsaturated fatty acids, low concentration of antioxidants, high rate of oxygen consumption, and availability of redox-active iron [57]. In addition, differences in relative activities of the antioxidant enzyme systems in the mature and immature brain results in differences in the response to oxidative stress after brain injury. Superoxide dismutase (SOD) existing as Cu,Zn-SOD (SOD1) in the cytoplasm and Mn-SOD (SOD2) in the mitochondria, catalase and glutathione peroxidase (GPx) are the cell´s most important antioxidant defense mechanisms. SOD1 and SOD2 convert O_2- to H_2O_2 while catalase and GPx scavenge H_2O_2. SOD levels are low embryonically and increase with increasing postnatal age, following changes in oligodendrocyte maturation and myelin sheath synthesis, while glutathione peroxidase remains relatively constant after birth [58]. More importantly, the response of these antioxidant enzymes to oxidative stress also appears to be different in the neonate compared to the adult. For example, SODl overexpression worsens HI injury in neonates instead of diminishing the damage. Neonatal mice transgenic for SOD1 had greater brain damage than their non- transgenic littermates. [59]. This lack of benefit from increased SOD1 appears to be due to the accumulation of H_2O_2 seen in the neonatal brain compared to the adult where overexpression of SOD1 is protective [59–62]. Increased vulnerability of the immature brain to accumulated H_2O_2 coupled with an inability to adequately scavenge the accumulated H_2O_2 by increasing antioxidant activity is likely responsible for the greater susceptibility to oxidative stress in the neonatal brain [59–62]. The neonatal brain is also more prone for excitotoxic injury because expression of the glutamate

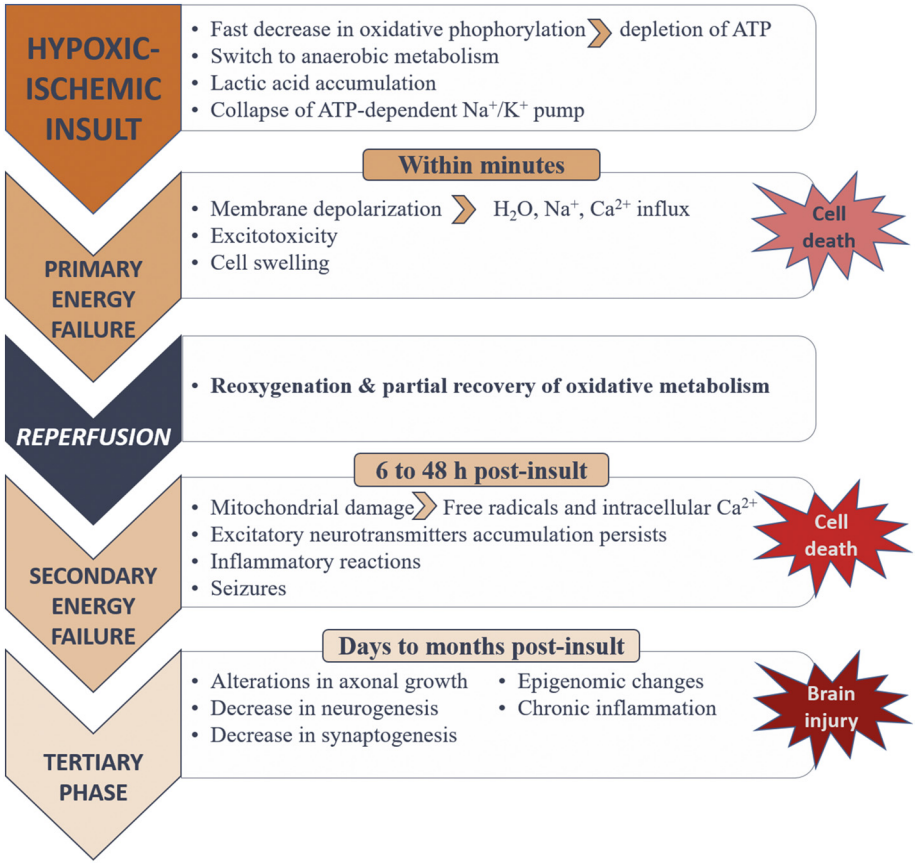

Fig. 1. Pathophysiological mechanisms of neonatal brain injury after hypoxia–ischemia in term neonates. Immediately after severe hypoxic–ischemic insult, primary energy failure takes place. Secondary energy failure occurs after reperfusion and reoxygenation, during the following 6 to 48 hours. Lastly, the tertiary phase happens, where injury perpetuates along with chronic inflammation (from days to months).

receptors N-methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is greater in the neonate than the adult [63]. Developmental changes in microglial phenotype, distribution and numbers can also increase susceptibility of the neonatal brain to inflammatory insults. A transient increase in amoeboid microglia in the white matter tracts is normally seen in the fetal and perinatal period subsequently changing to a more ramified microglial phenotype that is mostly in the cortex at older ages and in adults [64]. This may also explain the increased vulnerability of the periventricular white matter in the immature brain when compared to adults.

3. Growth factors involved in neuroregeneration

The damaged newborn brain has a limited capacity to adapt and regenerate after a deleterious event such as HI. In neonates, a hypoxic-ischemic insult can detrimentally alter the cellular composition of the neurovascular niches [65]. Although proliferative processes in the SVZ and SGZ are maintained, adaptive functional changes take place which lead to impairments in neuronal commitment, integration and functionality and consequently impede complete repair [66]. It is therefore crucial to find ways to assist the brain's endogenous regeneration efforts [67].

To be able to manipulate the endogenous neural progenitors it is crucial to have knowledge of the extracellular signals that can stimulate cell division and regulate the fate of neurons, glia and progenitor cells. Several factors can help increase postnatal neurogenesis by stimulating the formation and/or enhancing the survival of new neurons. Neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), the neurotrophins NT3 and NT4-5, glial-derived neurotrophic factor (GDNF), as well as mitogens such as the epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), insulin-like growth factor-1 (IGF-1), sonic hedgehog (Shh) and finally erythropoietin (EPO), are delivered through blood or the CSF or released by surrounding cells and are essential for the survival and differentiation of normally developing neurons, while also playing a pivotal role in the protection and recovery of mature neurons under pathologic conditions.

3.1. Role of the neurotrophin family

BDNF and NGF, along with the small proteins NT-3 and NT4-5 have been designated as the family of neurotrophins [68]. The developmental significance of neurotrophins is reflected in the timing of increase in their expression coinciding with the onset of neurogenesis. Their site and stage specificities vary greatly. In particular, NT-3 and BDNF display reciprocal patterns of expression with NT-3 expression being the highest in newborns and especially in the most immature areas of the brain, where BDNF expression is minimal. On the other hand, BDNF is higher in neurons that mature earlier such as in the midbrain [69]. Their distinct actions are reflected in the binding of their cognate receptors, with BDNF and NT-4 acting through tyrosine kinase receptor B (trkB) and NGF through trkA. NT-3 acts through trkC but can also activate trkA at much higher concentrations than does NGF. All neurotrophins can bind p75NTR with similar affinity. Neurotrophins have usually been associated with the concept of neuroprotection, like continuous low-dose infusion of NT-3 or BDNF into the striatum for 3 days prior to HI induction proved protective for striatal medium spiny neurons [70]. However, their function includes neuroregeneration, revascularization and anti-inflammation [71].

BDNF signaling plays a pivotal role in learning, memory and neuroplasticity and is likely to be required for long term survival of newborn neurons [72]. Its neurogenic effects are mediated through two distinct receptor pathways: the p75 neurotrophin receptor (p75NTR) pathway and the tyrosine kinase receptor B (trkB) pathway. BDNF protein expression is particularly high in the hippocampus during brain development and it is essential in promoting synaptic plasticity within the hippocampus and the prefrontal cortex, regions especially important for memory consolidation [73,74]. BDNF is known to be implicated in the pathophysiology of a wide range of neurological diseases including neonatal HI. Upregulation of BDNF was observed in the serum and CSF of neonates with HI [75]. Furthermore, early measured BDNF levels have been positively correlated with the severity of damage on magnetic resonance imaging in newborns with HI. In contrast, at a later stage, the sustained elevation of BDNF was assumed to be reflective of the neuroprotective and reparative processes taking place in the brain [76,77]. BDNF has been shown to display neuroprotective effects when administered intraventricularly both in adult rats following permanent middle cerebral artery occlusion (MCAO) and in neonatal rats following HI injury (unilateral carotid ligation followed by hypoxia) [78,79], BDNF also led to enhanced neurogenesis after intravenous injection in an adult photothrombotic cerebral ischemia model [80].

Closely related to BDNF is NGF, whose high specificity restricts its effects to sympathetic neurons, neural-crest-derived sensory neurons and striatal and basal forebrain cholinergic neurons. Just like BDNF, it acts through p75NTR and tyrosine kinase receptor A (trkA). These signaling pathways have been known to be broad based, dynamically regulated and context dependent. Many neural, nonneural and glial cells alike, express NGF receptors and diverse signaling cascades are triggered in response to interacting with them [81]. In the context of neonatal HI, administration of NGF has appeared to be globally neuroprotective to the developing brain [82]. When administered to two children suffering from severe hypoxic-ischemic brain injury it improved their consciousness level and communicative functions which is congruent with NGF's effect on cholinergic neurons [83].

3.2. Role of Glial derived neurotrophic factor (GDNF) regeneration

GDNF was first identified as a survival factor for dopaminergic neurons [84]. As a member of the TGF-β superfamily, it is known to be one of the most potent neurotrophic factors. In neonatal rats with HI injury, GDNF levels were upregulated within 48 hours and were associated with reduced apoptosis, indicating that GDNF may decrease neonatal brain injury [85]. Furthermore, in a related study GDNF's ameliorative effects were speculated to be derived from its ability to protect neurons from oxidative stress. In the same study a single intracerebral injection of GDNF within 30 minutes from the insult was able to reduce infarct size in a time dependent manner [86].

3.3. Mitogens and other factors that promote cellular regeneration

EGF and FGF-2 both have a strong mitotic effect on SVZ neural stem cells and progenitors. Their receptors are actively expressed in the infant, juvenile and adult human SVZ [87] as well as in actively dividing cells in the postnatal rodent SVZ [88]. Signal transduction through these receptors is important for their proliferation and self-renewal during embryonic and postnatal development. Subsequent to HI brain injury, there is a regenerative response elicited from the stem and progenitor cell pools within the SVZ and demonstrated by an overexpression of the EGF receptor [89]. FGF-2 which is also known as basic fibroblast growth factor (bFGF) increases the levels of a molecule called neurogenin-2 (NEUROG2), a proneural transcription factor that is required for neural differentiation [90]. The effect of FGF-2 increased the number of newborn neurons in the olfactory bulb, the destination point of SVZ progenitors during brain development [91]. There are progenitor cells responding to EGF and other growth factors outside the SVZ. These interactions are mostly seen in the mature brain [92]. Similarly, targeting EGF receptors on progenitor cells with recombinant human heparin binding EGF by intranasal delivery, after hypoxic insult to the developing brain resulted in the regeneration of oligodendrocytes from progenitor cells leading to functional recovery [93].

IGF-1 is abundantly expressed throughout brain development. Blood-borne IGF-1 can enter the brain and regulate the functions of the cells comprising the blood-brain barrier as well as the cells making up the functional unit of the brain such as oligodendrocyte progenitor cells and neurons. This is considered a key element in the neurobiology of IGF-1 which is also involved in modulating brain plasticity and brain excitability [94,95]. Serum IGF-1 level has been reported to decrease in human neonates suffering from HI brain damage [96]. The same observation has been made in a rodent animal model [97]. In postnatal day 7 rats with HI injury, immediate intraventricular infusion of IGF-1 resulted in a reduction of HI brain injury [98] whilst delayed subcutaneous administration was able to produce a sustained functional recovery [97]. Combination of intranasally administered IGF-1 and hypothermia treatment in rat pups attenuated brain damage [99] whereas intranasal IGF-1 alone was only successful within a therapeutic window of 1h after the insult [100]. The neuroprotection provided by IGF-1 has been attributed to antiapoptotic and mitogenic effects particularly on immature oligodendrocytes [101,102].

A potentially important factor for hippocampal neurogenesis is the potent mitogen Shh. Its receptor, Patched, is highly expressed in the DG of the hippocampus of adult rats as well as in progenitors isolated from that area. Shh signaling seems to be required in the mammalian telencephalon during development in order to maintain the population of neuronal progenitors and promote their proliferation and differentiation towards neurons and oligodendrocytes [103]. Wang *et al.* [104] administered umbilical cord blood (UCB) mononuclear cells as a treatment strategy to HI rats and demonstrated an augmented proliferative effect on neural progenitors that was mediated through Shh signaling. Moreover neural progenitors and neurons upregulate Shh expression in response to hypoxia but this effect is only limited in the hippocampus [105].

Finally, EPO, an inducible cytokine, was originally identified for its role in erythropoiesis but is also activated during the intrinsic response of the brain to hypoxia. Being capable to regulate a hypoxia attributable neuronal production, EPO plays a vital role in neurogenesis and differentiation, controlling the multipotent NSC numbers that restrict to neuronal lineage [106]. EPO exerts an antiapoptotic effect in a variety of different models of ischemic brain injury. In neonates, it has been found to reduce infarct volume, to improve short term sensorimotor and long term cognitive functions [107,108] while late administration, enhanced white matter recovery and oligodendrogenesis [109]. Furthermore, experimental rodent studies using neonatal stroke models have had similarly encouraging results [110]. Currently, several clinical trials for EPO are underway such as the HEAL study (NCT02811263), the NEATO study (NCT01913340) and the PAEAN study (NCT03079167) combining EPO and hypothermia treatment.

Other factors with potentially neuroregenerative properties that have not received as much attention include persephin (PSP), a member of the TGF-β family and closely related to GDNF, hepatocyte growth factor (HGF) which mainly regulates angiogenesis but also has antiapoptotic, neurogenic and antifibrotic abilities [111,112], ciliary neurotrophic factor (CNTF) and granulocyte macrophage colony stimulating factor (GM-CSF).

3.4. Role of vasculature in regeneration

Another aspect that is crucial for the survival and development of new neurons is the provision of adequate blood supply. The vasculature provides nutrients to the NPCs and regulates their proliferation and migration. The SVZ vascular plexus is entirely unique for that area, in a way that supports stem cell proliferation and regeneration. Blood

vessels lack astrocyte and pericyte coverage, allowing for the proliferating cells to directly receive special cues and signals derived from the blood. Such distinct architecture highlights the importance of the vascular system in regulating neurogenesis and regeneration [113].

Opposite to the relatively stable neurovascular bed of the SVZ, in the SGZ of the hippocampus a large percentage of the proliferating cells are endothelial cells. In the hippocampal neurogenic niche, angioblasts and neuroblasts proliferate together in "clusters", simultaneously, while sharing or depending on the same signaling processes. It is possible that signaling molecules that act on both populations of cells regulate neurogenesis in that area [114].

FGF-2 in particular has a recognized role as a mitogen for neural stem cells but acts at the same time as a strong angiogenic factor [115]. Furthermore, a synergistic loop involving FGF-2 and vascular endothelial growth factor (VEGF), where endothelial responses to VEGF are dependent on FGF-2, may initiate the proliferative phase of the neural precursors of SGZ [116]. Conversely, an increase in the circulating VEGF and thus angiogenesis, may bring forth neurogenesis, since the provided trophic support for the immature neurons assists their long-term survival. Finally, it has been demonstrated that VEGF acts directly as a trophic factor for neurons [117,118].

Finally, neurogenesis in the postnatal brain only occurs at the unique anatomical interfaces that are the SVZ and the SGZ, and it highlights the significance of neurovascular interactions. Therefore, strategies that aim in boosting angiogenesis may also prove valuable for brain injury therapeutics. For an extensive review of the exact signaling pathways that regulate neurogenesis in the SVZ and SGZ niches (Notch, Wnt signaling pathways etc.) see Ruddy *et al* [119]. Pre-clinical and clinical evidence for the benefits of administration of EPO, BDNF, VEGF and IGF-1 in treating different causes of brain injury is analytically provided by Larpthaveesarp *et al.* [120].

4. Stem cell-mediated gene therapy

During the last decade, cell-based therapy has evolved in a way that enables the production of "designer" cells for the treatment of neurodegenerative disorders. Cell based therapy with the utilization of stem cells has long been investigated as a very appealing treatment strategy and using cells as vehicles to establish a local infusion of growth factors and proteins may boost neurogenesis. Using viral vectors, therapeutic genes of interest can be incorporated into the stem cells *ex vivo* and then transferred to the host tissue by transplantation, thus preventing exposure of the host to the vector with potentially detrimental effects [121].

Enhancing stem cell function with the insertion of genes can augment their therapeutic benefits. The interaction of genes and cells may prove synergistic, ensuring stable and efficient expression of the therapeutic gene, and further improving the behavior and survivability of the cells by autocrine or paracrine influence. This approach could also prove valuable in supporting the endogenous repair efforts by enhancing angiogenesis or modifying the post-injury microenvironment, making it favorable for the survival and integration of newly generated neurons [122]. Therefore, the use of cells ensures the prolonged secretion of the desired growth factor, achieving better effects than with one single intervention, as opposed to administering growth factors alone.

Finding an efficient and noninvasive delivery route has always been an important aspect of developing cell treatments for cerebral ischemia. Conceptually, local administration would be the most direct method to consider. This includes intraparenchymal transplantation, intrathecal or intraventricular injection. The invasiveness of such procedures though significantly limits their clinical application. Reports have shown that systemically injected stem cells can replicate the neurologic benefits of intracerebral transplantation [123]. However, a major problem with systemic introduction is the so-called 'pulmonary first-pass effect', resulting in a large percentage of the cells being trapped within organs other than the brain and more specifically, implicating pulmonary passage as a major obstacle between the transplanted cells and the injured brain. As a result a therapeutically questionable number of cells reach the brain [124]. Higher efficiency of stem cell engraftment has been achieved with intra-arterial infusion [125,126]. Still the intra-arterial and intravascular transplantation routes have been associated with pulmonary embolism in laboratory small animals [127]. This complication is especially seen with mesenchymal stem cells derived from the adipose tissue [128]. Recent studies have had success with administering cells intranasally. The reliability of this method has been shown in rodents, however it's utility in stem cell administration to humans has not been determined yet. Nevertheless, it has clear advantages over the classical routes as it is less aggressive, bypasses the blood brain barrier and there is less systemic exposure. At present, there is evidence for the validity of the direct nose-brain delivery route to humans with a wide range of therapeutic agents [129,130].

The risk of immunological rejection has posed many constraints in the application of stem cell therapies in clinical practice. In allogenic stem cell transplantation, stem cells are collected from a matching donor and transplanted into a patient. Similar to whole organ transplantation, administration of allogenic stem cells is always accompanied by a long-term dependence on immune suppression to avoid the harmful complications of the graft-versus host disease (GVHD) [131]. On the other hand, deriving stem cells from patients in an autologous manner promises to bypass the immunological consequences of using allogenic stem cells as a source for cell therapy. However, autologous stem cell transplantation is difficult to attempt because of a cell preparatory period and cell transplantation timing.

Finally, malignant transformation is a potential risk with any cell therapy. The risk is particularly high with embryonic stem cells and induced pluripotent stem cells [132] and is far less with adult stem cell tissues [133].

Most experimental efforts with stem cell mediated gene delivery for ischemic brain injury have been done using tissue-specific adult stem cells (neural or non-neural). Below we discuss the three main types that have served as "seed cells" for gene therapy targeting adult and neonatal stroke: neural stem cells (NSCs), mesenchymal stem cells (MSCs) and UCBs.

4.1. Neural Stem Cells (NSCs)

Neural stem and progenitor cells, collectively termed as Neural Stem Cells (NSCs) can be derived from the embryonic or fetal brain but also reside in the SVZ and the SGZ of the dentate gyrus of the adult hippocampus. They are operationally defined by their ability to self-renew and differentiate into cells of glial and neuronal lineages. Stroke studies using embryonic stem cells have been faced with the challenge of difficult procurement and the risk of tumorigenesis. For brain injury, adult NSCs have been employed the most, given their clear potential to differentiate into neural cell lineage [134].

NSCs are thought to exert benefits through several mechanisms. Despite the initial fascination over their theoretical potential to restore dead circuitry by directly replacing the damaged cells, the current preponderance of evidence suggests that direct cell substitution is not the primary mechanism of action of these cells [135]. It is presently well accepted that NSCs produce a wide range of paracrine neurotrophic factors (BDNF, NT3, NGF, CNTF [136]) and seem to have a strong tropism for central nervous system (CNS) pathology, meaning that they can shift their differentiation fate in response to the local microenvironment [137]. Their inherent ability to develop into cytoarchitectural components of the host brain such as neurons, astrocytes, oligodendrocytes or even quiescent progenitors, circumvents many of the limitations of viral vectors in treating widespread lesions of the brain.

Several studies with NSCs directed to treat neonatal HI have focused on stem cell mediated angiogenic gene transfer and more specifically, on the transduction of VEGF. Aside from the fact that VEGF has strong neurotrophic and angiogenic involvement, it is one of the target genes

of hypoxia inducible factor-1α (HIF-1α) and is in big part responsible for the ischemic tolerance seen in hypoxic preconditioning [138,139]. Zheng *et al*. have demonstrated that causing overexpression of VEGF with adenoviral vectors in the brain of neonatal rats can be neuroprotective [140]. The same group later on used a recombinant lentiviral vector containing the gene $VEGF_{165}$ to transfect rat NSCs *ex vivo* and then injected the cells into the sensorimotor cortex of newborn rat pups 3 days after the induction of a hypoxic ischemic injury to model cerebral palsy. Their strategy led to an increase in VEGF levels detected in the brain and recovery of sensorimotor function of the treated rats [141]. In a separate study, newborn animals received NSCs transfected with the same VEGF gene using the same protocol. The researchers observed that animals treated with the transgene NSCs had marginally improved brain structure and motor skills compared to animals that received naïve cells [142]. The same results were reproduced by Yao *et al.* who reaffirmed the superiority of transgene NSCs in protecting the brain from neuronal loss and in promoting functional recovery versus normal NSCs [143].

Lee *et al.* undertook a more direct approach to neurogenesis by exploring the potential of neurogenin-2 (NEUROG2), a proneural transcription factor, that is expressed during development and is one of the determinants of the identity transitions that decide neural cell fate [90]. Human NSCs expressing the NEUROG2 gene were injected directly in the infarcted area of HI injured neonatal mice. NEUROG2-NSCs favored differentiation into astroglial lineages and stimulated neurite outgrowth, cell survival and provided trophic support through the secretion of growth factors. In this study as well, NEUROG2-NSCs contributed more efficiently in the regeneration of the damaged regions than their naïve NSC counterparts [115,136].

Murine NSCs have also been transduced with the neuron-inducing growth factor NT3, which is inherently expressed in low amounts by the NSCs. A retroviral vector was used for the insertion of the NT3 gene in cultured NSCs and the cells were directly injected into the infarcted region of HI damaged mice. The endogenous expression of NT3 was enhanced in the genetically engineered NSCs and their differentiation was directed toward neurons at the expense of gliogenesis, which is the pathway in transduced NSCs [144].

Recently, Ye *et al.* used an adenoviral vector to infect NSCs with the neurotrophic factor bFGF (alternatively known as FGF-2) which is a central mitogen with effects on a large panel of cells and tissues, encouraging the proliferation, differentiation and migration of endothelial cells and neural progenitors alike [91]. NSC-bFGF cells were administered intranasally to neonatal HI injured mice and were observed at the lesion site, but not at the contralateral hemisphere. While NSCs are generally not known to drastically alter the microenvironment, the design of NSCs carrying the multipotent bFGF gene minimized myelin and gray matter loss and ameliorated impairment in motor performance of the recipient mice [145].

4.2. Mesenchymal Stem Cells (MSCs)

Devoid of the ethical concerns associated with embryonic stem cells, adult MSCs have captured the scientific interest over the last four decades and have been considered as an attractive vehicle for gene therapy [146,147]. These spindle-shaped fibroblast colony-forming cells feature a number of intrinsic and environmentally responsive properties that have delineated them as the seed cells with the greatest plasticity amongst all other stem cell types [148]. In addition to self-renewal and proliferation, their progeny can differentiate, in a controlled manner, into cells of all three lineages [149]. In specific differentiation media MSCs can give rise to chondrocytes, adipocytes, osteocytes [150], hepatocytes [151], cardiomyocytes [152] or neurons [153]. MSCs are further capable of systemic migration and unlike neural stem cells, they have not yet been associated with tumorigenesis [154]. Perhaps, the most important attribute of MSCs has been their immunosuppressive profile [155] which renders them 'invisible' to the host's immune system response enabling transplantation without the need for tissue matching [156]. Their immunomodulary properties render these cells refractory to rejection and they have been repeatedly shown to nonspecifically suppress GVHD during allogenic transplantation [157–159]. Finally, MSCs have been acknowledged for their considerable tolerance of genetic engineering manipulations, without any sign of structural aberration of their genome [122].

Just like NSCs, it was once speculated that MSCs aid in tissue regeneration through homing to the site of injury and directly differentiating into the tissue to be generated as they have been reported to differentiate into neural elements [160,161]. Yet, the leading theory now is that MSCs act through the functional secretion of bioactive factors [162], including colony-stimulating factor 1, stem cell factor, VEGF, bFGF, NGF, BDNF and so forth [163]. Although the two mechanisms of action (paracrine effect of MSCs and cell replacement by MSC-derived cells) do not necessarily negate each other, the former concept can be exploited by transforming the cells into "biological minipumps" at the implantation site. Administration of naïve stem cells or growth factors alone has already delivered positive outcomes on lesion size and cognitive or motor recovery [80,164].

Despite the promise of MSCs, the use of naïve stem cells has been met with several limitations. These include the long culture period that is often required in order to obtain a sufficient number of cells for transplantation, challenges with ensuring purity of the isolated MSC populations, their inducibility towards specific phenotypes and their compromised homing efficiency. Thus, it appears desirable that the differentiation and migration of both donor and host cells might be enhanced with the *ex vivo* genetic manipulation of MSCs for specific transgenes like growth factors.

The most conventional genetic modification that has been attempted with MSCs is the conjugation of the BDNF gene. As is discussed in the previous section, BDNF has a vital role in the development of the nervous system and has been shown to have neuroprotective effects. Intranasal administration of BDNF-hypersecreting MSCs at 3 days post MCAO led to long-term attenuation of motor deficits, dampened inflammation resulting in decreased gray matter loss and lesion size, and cell proliferation in the SVZ but the researchers concluded that MSC-BDNF and naïve MSCs behaved comparably with respect to these results [165]. In a following study by the same group, MSC-BDNF were compared to MSCs modified to secrete epidermal growth factor-like 7 (EGFL-7), Shh and PSP. MSC-BDNF brought forth similar outcomes, improving motor function, reducing the lesion size and inducing sustained proliferation in the hypoxic ischemic hemisphere. These effects were much more prominent comparing to the other stem cell groups. Treatment with MSC-EGFL-7 only promoted proliferation of co-cultured NSCs without affecting differentiation *in vitro*. MSC-PSP did not have any significant effect comparing to naïve MSCs. Finally, the presence of MSC-Shh stimulated the differentiation of NSCs toward neurons and astrocytes [166].

In animal models of adult ischemic stroke, studies have used MSCs that over-express many different neurotrophic factors and cytokines. BDNF overexpression is still the most common genetic modification seen, as is considered to be the most potent neurotrophic factor for neuroregeneration [122,167,168]. Some of the most noteworthy attempts with other genes that have shown promise in adult models of ischemic stroke include CNTF, NT3, GDNF [167,169], VEGF [170], angiopoietin-1 (Ang-1) [171,172], HGF [173], FGF-1 [174] and bFGF [175]. These therapeutic strategies may also be worth exploring in neonatal brain injury.

4.3. Umbilical cord - derived stem cells

Another very attractive non-embryonic source of stem cells is the umbilical cord. The umbilical cord blood contains numerous stem and progenitor cell types such as hematopoietic stem cells, MSCs and the unipotent endothelial progenitor cells. Among these cell populations,

only the umbilical cord blood MSCs (UCBs) possess neurogenic differentiation potential [176] and as such, they have been implemented widely in cell therapy strategies against neonatal HIE. UCB therapy is most likely the safest and most feasible among stem cell therapies since umbilical cord blood has been used in clinical practice for hematopoietic stem cell transplantation for decades [177]. UCBs appear to exert their neuroprotective effects through their immunomodulatory/anti-inflammatory action [178,179] or the reduction of apoptosis and oxidative stress [180–182]. Furthermore, they have been reported to provide a favorable environment for enhancing the brain's regeneration process through the secretion of various growth factors or their angiogenic / vascular reparative capabilities [183]. Numerous pre-clinical studies have demonstrated the therapeutic effect of administering human derived UCBs intraperitoneally [181,183–185], intra-arterially [126,182], intravenously [186,187] or intraventricularly [188]. Additionally, several clinical trials to test the feasibility and safety of autologous UCB IV administration have either been completed (NCT01649648) or are underway, such as the NEOSTEM study (NCT02881970) and others (NCT02612155, NCT02256618, NCT02612155).

There has only been one report of genetically transfected UCBs administered to neonatal rats with HI brain injury [189]. The authors referred to the transplanted cells as human umbilical cord-derived NSCs. Following the injection of IGF-1-transfected UC-NSCs into the tail vein of a neonatal rat model, the authors noticed increased cell proliferation and enhanced differentiation toward the neuronal lineage at 7 days post-transplantation. They also observed that the preservation of behavioral function was better than the control group, which had received an intravenously injection of naïve NSCs (Table 1).

UCBs are not the only UC- derived stem cells that have been investigated as alternatives for the treatment of neonatal HIE. In particular, MSCs isolated from the stroma of the umbilical cord (Wharton's Jelly - MSCs) are of special interest [190]. Just like the UCBs, they are available at birth for autologous transplantation and their use does not evoke the ethical concerns of embryonic stem cells, the rejection risk of allogenic stem cell sources or the tumorigenesis risk of induced pluripotent stem cells. Other advantages include the non-invasiveness of the collection procedure and the fact that the umbilical cord does not require a complicated culture process. A common issue with the umbilical cord blood is the limited number of nucleated cells that can be acquired. By contrast, Wharton's Jelly stem cells demonstrate a higher frequency of colony forming fibroblasts than either the UCBs or bone-marrow derived MSCs [191]. Despite their pluripotent possibilities, genetic manipulation of Wharton's Jelly stem cells for the treatment of neonatal HIE has not been described yet.

5. *In vivo* and *ex vivo* gene transfer therapies

Stem cell technology when coupled with gene therapy can evolve into a powerful tool for the management of perinatal brain injury. Making use of the steady advances in gene manipulation and interfering with specific pathophysiological or regenerative pathways will inevitably drive the care of infants with brain injury to take on a more personalized form.

Viral vectors have been successful vehicles for gene delivery thus far [192]. However, the design of a specific vector to carry the desired gene and the safety associated with their administration pose limitations on their clinical applications. The vectors available today are to an extent difficult to direct towards the specific cell types and regions that are in need of correction [193]. Retroviral vectors only affect mitotic cells [194], which are scarce in the post-developmental CNS and are usually not the cells requiring regeneration. Adeno-associated viral (AAV) and lentiviral vectors, while able to infect postmitotic cells [195], do not often target large regions and multiple cell types that need correction. AAV vectors, despite possessing a wider distribution, may display selectivity for neurons to the exclusion of glia [196–198]. One of the inadequacies of most existent gene therapy techniques is that they aim to insert new genetic information into old neural substrates, which may already have become dysfunctional or degenerate at the injury site [199]. The need is for the development of new substrates where the therapeutic genes can operate [200].

In this context, stem cells and young neurons seem to be the ideal graft material for gene delivery. As compared to the classic *in vivo* gene therapies using viral vectors, in *ex vivo* therapies there is no exposure of the patient to the gene transfer vector and the cells that are being transduced can be selected, expanded and differentiated before or after the gene transfer to maximize their potency and safety [121,192]. This, however, requires a thorough understanding of the tropism mechanisms of the stem cells targeting the injured brain. The expectation that cells implanted or migrating into damaged areas will direct replacement of a wide variety of cell types is unrealistic. Therefore, it is critical for the epigenetic modifications that will take effect, to be able to orchestrate every aspect of the neural development including brain patterning, neural stem cell maintenance, neurogenesis, gliogenesis and synaptic and neural network connectivity and plasticity. The specific factors and conditions that are most responsible for optimizing the odds with cell therapy have not yet been delineated.

Moreover, several other important hurdles must be overcome to ensure satisfactory delivery, feasibility of translation to the clinic and safety to use in a variety of clinical settings. Principally, although the probability is lower with MSCs, the risk of malignant transformation of the genetically modified transplanted cells or tissues that have been under the influence of the secreted factors is constantly present [201]. Other problems to be addressed include the toxicity of the transplantation itself which is always an issue even with naïve stem cells, as well as ensuring that the observed functional and histological benefits will remain intact long term.

Finally, gene therapy by itself incurs a delay between gene delivery and expression. When targeting the acute phase of brain injury, the intervention of stem cells as vehicles for gene therapy certainly renders it a lesser attractive option for neuroprotection, if one considers the extra time that is required for the cells to engraft, differentiate and produce the transduced gene products [193,202]. A further complexity is also the recognition that some factors may not be able to be engineered simultaneously because they may interact in an antagonistic manner within the cell or the microenvironment. Thus, careful planning and advance knowledge of how each stem cell type processes certain molecules are prerequisite [200].

The therapeutic efficacy of gene transfer mediated by stem cells might improve by incorporation of various combination strategies that will help extend the survival of the administered cells, modify the post-injury microenvironment and circumvent the irregular tissue gaps that develop after brain injury. A potential advance towards that would be the various biocompatible polymeric scaffolds especially in the case of large tissue defects [203]. As an alternative strategy to cell-based therapies, recent advances in nanotechnology may provide novel mechanisms for the delivery of bio-active factors specifically for enabling CNS regeneration following injury.

6. Nanoparticles for drug delivery in perinatal brain injury

Nanotechnology-based approaches are rapidly expanding as potential tools for the diagnosis and treatment of neurological disorders [204,205]. They can be designed to respond to specific cellular and molecular environments and can induce desired physiological responses, while diminishing detrimental side effects [206]. Furthermore, the bioavailability of therapeutics can be increased by using nanoparticles (NPs) for delivery through improved solubility, reduction of enzymatic degradation, decreased protein binding, evasion of clearance by reticuloendothelial system enabling controlled/extended release [207] and targeted delivery to the site [204]. Therefore, NPs are an emerging and revolutionary approach for delivery of therapeutics in neurodegenerative and neurologic diseases [208–211].

Table 1
Summary of genetically modified stem cells used in experimental models of neonatal stroke and hypoxic-ischemic (HI) injury (abbreviations: hypoxic-ischemic encephalopathy=HIE, MCAO=middle cerebral artery occlusion, mesenchymal stem cell=MSCs, neural stem cells =NSCs, insulin-like growth factor-1=IGF-1, brain-derived neurotrophic factor=BDNF, epidermal growth factor=EGF, sonic hedgehog=Shh, vascular endothelial growth factor=VEGF, neurogenin-2=NEUROG2, fibroblast growth factor=bFGF, postnatal day=P).

Disease model	HIE rat model, Left common carotid artery ligation, recovery 2 h, hypoxia: 8% O2 for 2 h	transient 1.5 h right MCAO	HIE mouse model, Right common carotid artery, recovery 1 h, hypoxia: 10% O_2 for 45 mins	HIE rat model Left common carotid artery ligation, recovery 2 h, hypoxia: 8% O_2 for 2 h	HIE rat model, Left common carotid artery ligation, recovery 2 h, hypoxia: 8% O_2 for 2 h	HIE rat model, Left common carotid artery ligation, recovery 2 h, hypoxia: 8% O_2 for 2 h	HIE mouse model, Permanent right common carotid artery occlusion, hypoxia: 8% O_2 for 1.5 h	HIE mouse model, permanent right common carotid artery occlusion, hypoxia: 10% O_2 for 45 min
Stem cell type	Human umbilical cord blood derived-NSCs	Rat MSCs	Mouse MSCs	Rat NSCs	Rat NSCs	Rat NSCs	Human NPCs	NSC line (C17.2 cell)
Growth factor/ Gene	IGF-1	BDNF	BDNF, EGFL7, Shh, PSP	VEGF	VEGF	VEGF	NEUROG-2	bFGF
Vector type	pcDNA3.1-IGF-1 recombinant plasmid- Transfection through liposome	Adenoviral	Adenoviral	Lentiviral	Lentiviral	Lentiviral	Adenoviral	Adenoviral vector with recombinant humanized GFP
Administration route	Injection into the tail vein	Intranasally	Intranasally	Injection into the left sensorimotor cortex	Injection into the left sensorimotor cortex	Injection into the left sensorimotor cortex	Injection into the center of the infarcted region	Intranasally
Concentration and dosing	1×10^6 cells once	10^6 cells once	5 x 10^5 cells at two doses, once	5 x 10^4 cells once	5 x 10^4 cells once	1 x 10^5 cells once	9.6 x 10^5 cells once	1 x 10^6 cells once
Administration time	24 hours after HI	3 days after MCAO	10 days after HI	3 days after HI	3 days after HI	3 days after HI	7 days after HI	3 days after HI
Animal type	Sprague Dawley rats	Sprague Dawley rat	C57Bl/6J mouse	Sprague Dawley rat	Sprague Dawley rat	Sprague Dawley rat	ICR mouse	ICR mouse
Animal age	P7, full term	P10, full term	P9, full term	P7, full term	P7, full term	P7, full term	P7, full term	P9, full term
Adjacent therapy or comparative therapy	-naïve NSCs	-naïve MSCs - vehicle (PBS)	- empty vector MSCs - vehicle - sham	- naïve NSCs - vehicle (PBS) - sham	- control group - vehicle (PBS) - naïve NSCs - sham	- vehicle (PBS) - naïve NSCs - sham	- vehicle (H-H buffer) -sham	- naïve NSCs - NSC-GFP - vehicle - sham
Main results	- Increased proliferation and higher expression of neuronal cell markers -Improved learning, memory and motor function	- Reduction of infarct size and gray matter loss - Early motor deficit improvement with the MSC-BDNF - Increased proliferation in SVZ - Differences between MSC and MSC-BDNF were not detected 28 days post-treatment	- MSC-BDNF improved motor function, induced long term proliferation of NSCs and reduced lesion volume both *in vivo* and *in vitro* -MSC-EGFL7 stimulated proliferation of NSC *in vitro* - MSC-PSP and MSC-Shh effects are no different than those of naïve (empty vector) MSCs	- Improved locomotor function - Increased growth rate - Decreased neuronal apoptosis - Increased microvascular density in cortex	- Increased expression of VEGF in HI injured animals - Higher VEGF levels in animals that received NSC-VEGF or naïve NSCs -Improved sensorimotor functions for both NSC groups - Alleviation of necrosis and degeneration for both NSC groups	- Improvement of sensorimotor deficits -Hippocampal cell loss was prevented for both NSC groups - NSC-VEGF performed better than naïve NSCs	-Engraftment and distribution of stem cells into the injured brain weeks after transplantation - Functional recovery - Protection against HI induced cytotoxicity - Increased neurite outgrowth and axonal sprouting	- Improvement of motor deficits without difference between the NSC groups - Increased differentiation into neuronal and glial lineages in the hippocampus and cortex in the NSC-bFGF group
Reference	Zhu et al, 2011	van Velthoven et al., 2013	van Velthoven et al., 2014	Zheng et al., 2012	Tan et al., 2014	Yao et al., 2016	Lee et al., 2016	Ye et al., 2018

6.1. Developmental considerations for designing nanoparticle platforms

6.1.1. Maturational effects that influence pharmacokinetics

One of the main advantages in using NPs as a drug delivery system to the CNS in the neonate and infant is to modify the pharmacokinetic properties of the drug, since drugs adopt the pharmacokinetic properties of the carrier [204]. Drug pharmacokinetics in the neonatal age group is often challenging due to the paucity of information and studies on metabolism and excretion of drugs in these age groups. It is well known that developmental changes related to maturation can affect absorption, distribution, metabolism and excretion (ADME) of drugs leading to a pharmacokinetic profile that is different than those seen in adults [212,213]. Nanoparticle platforms offer the advantage of limiting drug ADME that may be altered due to maturational effects. However, these aspects need to be also considered for the nanoparticle platform itself, depending on the type of platform and its surface modifications. For example, use of NPs for drug delivery may eliminate the need for enzyme, bile-acid or intestinal transporter related absorption which are all decreased in neonates [212]. However, the lower gastric acid secretion and relatively higher pH in the neonatal period, and decreased gastric motility in neonates [212,213] may influence nanoparticle absorption based on its surface modification which would need to be accounted for. Similarly, unlike most drugs, NPs may not be affected by the age-dependent maturation of Phase I or Phase II metabolism of the drug, since the nanoparticle platform will ideally enable delivery of the drug intact to the site of action. Renal clearance and glomerular filtration is highly influenced by maturation and both glomerular filtration and renal tubular functions are compromised in the neonate [214–217]. The physiologic low glomerular filtration rate in neonates can potentially lead to delayed renal clearance of the NPs. The low activity of organic anion transporters in the renal tubules in neonates [218] may alter transport and tubular secretion of anionic substances. Depending on the route of delivery and the site of action in the brain, all these aspects need to be considered before selecting and optimizing the appropriate NP platform.

6.1.2. Developmental changes in BBB that may have implications for NPs

In keeping with previous studies, the ideal nanoparticle platform for CNS delivery through systemic administration should not only have the standard characteristics of being non-toxic, non-immunogenic, and scalable but must also be able to bypass the blood-brain barrier (BBB), to penetrate brain tissue and to target specific cells if intracellular delivery is required [191–193, 201–203]. The main passage mechanisms across the BBB are transcytosis, endocytosis, transcellular and paracellular pathways, and a combination of these mechanisms. Charge and surface functionalities or surface modifications of NPs can influence their clearance, intracellular uptake and trafficking/cellular pathways [219]. Although systemic delivery of NPs to the CNS is challenging due to the need to overcome the BBB for delivery to the intact CNS, insults such as stroke and HI which lead to a temporary BBB disruption give an extraordinary opportunity for NP delivery to the injury penumbra [220]. Neonatal BBB and blood-CSF barrier have been shown to be well developed and functional since early embryonic states, similar to that in the adult brain, but the CNS barrier systems are not static during development as they change with brain maturation [221]. Transcriptome analysis of brain endothelial cells in mice has demonstrated several developmentally regulated brain endothelial signaling cascades and metabolic pathways. Pathways such as interferon signaling, growth hormone, circadian rhythm and phospholipase C signaling were upregulated in the neonatal endothelial cells while integrin signaling, protein ubiquitination and hypoxia signaling were downregulated in the neonatal compared to mature endothelial cells [222]. It is possible that these differences may dictate differences in responses to injury, however not much is known about the role of the BBB in perinatal disorders [223]. The presence of an injury can result in impairment of the BBB in neonates and the extent of impairment may vary based on the gestational age. Neonatal HI increases BBB permeability to small and large molecules within hours after the insult, which normalizes in the following days in animal experimental models [221,224]. After an insult, excitatory amino acid neurotransmitters are released, causing ROS-dependent changes in BBB permeability that allow immune cells to enter and stimulate an inflammatory response [223]. As in adult ischemia, the transient opening of the BBB after HI may exacerbate the damage but at the same time gives an opportunity to deliver therapies using NPs to the infarcted areas in the brain [224]. Therefore, alteration of the BBB appears to facilitate increased accumulation of the NPs in the brain, which may also depend on the type of disease, the developmental age and timing from the insult [225].

6.1.3. Maturational changes in extracellular space and proteins

After crossing the blood brain barrier, NPs must then move through the brain parenchyma in the extracellular space (ECS). The ECS' width is about 20% volume of the adult brain and may be higher in the newborn brain [226]. The ECS in the adult brain can accommodate globular particles up to 114nm [227,228] and ECS volume can decrease to as low as 5% after ischemia [229]. There are also changes in the extracellular matrix (ECM) proteins with development that may influence particle movement. There is differential regulation of the hyaluronan-binding proteoglycans in the developing brain based on their role in brain development. Among these, aggrecan and brevian, two of the chondroitin sulfate proteoglycans, steadily increase with age plateauing in adulthood while versican and neurocan are high in the postnatal period and decrease to adult levels rapidly [230]. ECM components such as Tenascin R and CSPG demonstrate regional and age-specific variations in human tissue dependent on the maturational events influencing cortical development [231,232]. It is possible that age and injury specific changes in the content of the ECM may also influence the movement dynamics of the NPs in the brain parenchyma. These factors need to be taken into consideration for designing the NPs for delivery of drugs and biologics to the neonatal brain. Since ECM proteins are known to influence neurogenesis and migration [233], delivery of drugs specifically to the ECS by NPs at critical time periods during development and after injury may be a strategy that can be utilized to promote neuroregeneration.

6.1.4. Maturational changes in microglia

The microglial response following injury to the neonatal brain usually involves the white matter since microglia are normally present in abundant numbers in the white matter tracts at this age unlike the adult brain where microglia are mostly in the cortex [64,221,234]. Any change in the microglial phenotype will alter normal development, and targeted delivery of drugs to attenuate microglial activation using NPs has shown to be a successful mechanism to enhance normal white matter development in different etiologies of neonatal brain injury [235–237]. In NE, there has been a recent focus on understanding endogenous neuroprotection and how to boost it or to supplement its effectors therapeutically once damage to the brain has occurred [18]. Targeting the tertiary damage phase might result in an improvement of long-term neurological outcomes in neonatal brain injury [53]. Likewise, there is a growing body of literature to suggest that strategies to target neuroinflammation can potentially decrease progression and increase the therapeutic window in neurodegenerative diseases [225]. When considering treatments for tertiary brain injuries, we would need to distinguish between strategies aimed at extending the window of therapeutic intervention from the acute phase to the subacute phase and strategies targeting more long-term events such as chronic inflammation or post-lesional plasticity [54]. The type of nanoparticle platform used and the therapeutic targets will vary based on the type of injury and the cellular response of the brain.

6.2. Nanotherapies evaluated in neonatal brain injury

The different nanotechnology strategies that have been evaluated for therapeutic applications in neonatal brain injury have been classified based on the pathophysiology of the neonatal brain injury and have been described below. A list of published nanoparticle-drug conjugates along with their targets, which demonstrate efficacy related to regeneration in models of neonatal brain injury are provided in Table 2.

6.2.1. Nanoparticles used in neonatal hypoxic-ischemic encephalopathy

As we have previously mentioned, TH is the standard clinical care for term newborns with moderate to severe NE in high-income countries [16,17]. However, a large percentage of the treated newborns still have an adverse outcome after injury and TH is not feasible in low- and middle income countries [4], so additional and adjunct therapeutic strategies are urgently needed. Clinical trials with melatonin [238], xenon [239], erythropoietin [240–243] or stem cell therapy [244,245] are currently ongoing. Additionally, there are other compounds in preclinical development, including N-acetyl-l-cysteine (NAC), lithium and polyphenols (resveratrol and curcumin) [55,246]. Delivery of all of these compounds can be improved by the use of carefully designed nanoparticle platforms.

NPs have been evaluated for delivery of neuroprotective agents in both preterm and full term rodent models and in a piglet model. The induced brain damage ranged from mild, moderate/severe to severe. The neuroprotective agents evaluated were recombinant erythropoietin (r-EPO) nanoformulation with poly-DL-lactide-coglycolide (PLGA), NAC conjugated to generation-4 hydroxyl (G4-OH) poly(amidoamine) (PAMAM) dendrimer labeled with the fluorescein Cy5 (Cy5-D-NAC), curcumin loaded poly(lactic-co-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) and quercetin nanosomes with lecithin/cholesterol/2-hydroxypropyl-beta-cyclodextrin (2HBCD). The anti-inflammatory and antioxidant properties of these agents are well known [247–250].

EPO was significantly more effective as r-EPO-PLGA nanoformulation, because of improved stability and controlled release with better delivery across the BBB [251]. Nanoerythropoeitin administration post-insult not only reduced infarction volume 72 h after the insult but also increased long-term motor ability, evaluated by rotarod at 21 days after HI in full term rats. Hence, when compared to free r-EPO, nanoerythropoetin was much more effective at lower dosages indicating the beneficial effects of NPs as a delivery vehicle [251].

In a mouse model of neonatal HIE, intravenous administration of Cy5-D-NAC was found to target activated microglia, astrocytes and injured neurons. Most importantly, Cy5-D-NAC uptake was not affected by hypothermia indicating that it can be used in combination with TH for treatment of neonatal HIE [252]. Since TH is known to alter the disposition and metabolism of pharmacological interventions [253–255], it is possible that it may also potentially slow down active cellular uptake mechanisms that are necessary for intracellular delivery of drug by NPs. The study by Nemeth *et al.* demonstrated that TH does not affect dendrimer uptake, and therefore therapeutics may be delivered by dendrimers in combination with TH without substantially changing the biodistribution of the dendrimer NP [252]. The authors also examined the effects of time of administration on dendrimer uptake and found that it was not a deciding factor. Extent of dendrimer uptake correlated with the severity of injury and this correlation was strongest for microglial cells [252].

In another rodent model of HIE, systemically administered curcumin-loaded PLGA-PEG NPs demonstrated significant neuroprotection in neonatal rats [256]. Curcumin's poor bioavailability due to its hydrophobic nature and rapid metabolism in the liver were overcome by utilizing the NP formulation. When loaded in PLGA-PEG, curcumin was able to bypass the BBB, the brain parenchyma and localize specifically in the injured regions. The PLGA-PEG NP demonstrated a 10-20 fold increase in diffusivity in the cortex and thalamus of the neonatal brain when compared to PLGA alone, demonstrating that the "stealth" nature of the PEG coating enabled/enhanced rapid diffusion due to decreased interactions of the NP with the extracellular proteins. Curcumin-loaded PLGA-PEP NPs significantly reduced edema in HIE and the neuroprotective effect was most pronounced in the penumbra area and in the less severely affected regions. There were also some indication that PLGA-PEG alone may also have some therapeutic effects, which was felt by the authors to be due to the ability of PEG to suppress ROS production. PLGA-PEG/curcumin reduced microglial activation and the number of amoeboid morphological cells, but only closest to the regions of neuronal injury.

Similarly quercetin, another polyphenol, when delivered intravenously using a lecithin/cholesterol/2HBCD nanosome formulation was shown to improve brain function and hemodynamic instability after severe hypoxia in newborn piglets [257]. Quercetin nanosomes treatment led to recovery of spontaneous breathing, suckling and ability to walk. However, this was not clearly associated with an improvement in pathology scores. These studies indicate that NP formulations help overcome issues such as drug solubility and enhance brain delivery.

6.2.2. Nanoparticles in periventricular leukomalacia or white matter injury

Therapeutic agents loaded to NPs have also been tested for the treatment of periventricular white matter injury and the evaluated drugs, NAC and EPO, are the same ones that have been tested for HIE. NAC conjugated to PAMAM dendrimers (D-NAC) when administered at either sub-acute or delayed time points was found to be protective in a preterm mouse model of ischemia-induced neonatal white matter injury [236]. D-NAC attenuated the pro-inflammatory response by reducing microglial activation while improving myelination. Cellular biodistribution of the dendrimer was based on the time of administration after injury and therefore the pathophysiologic response at the time point, with greater distribution in astrocytes in the acute phase and more in microglia at the delayed time point.

Likewise, when erythropoietin-loaded oligochitosan/sodium tripolyphosphate (OCS/TTP) nanoparticles (EPO-NPs) were subcutaneously administered for the treatment of PVL in a rat model induced by the administration of a toxin 3-nitroproprionic acid, an improvement in brain injury was noted [258]. EPO-NPs significantly attenuated lipid peroxidation and nitrite concentration, restored SOD and catalase enzyme activities, and led to protection of immature oligodendrocytes and preservation of myelin basic proteins. Similarly, when EPO-NPs were administered IP 1h after the injury in the same rat model on P5, liquefaction of the brain tissue was attenuated and those rats developed good memory and agility. EPO-NPs were effective at a dose that was ten times lower that of free EPO, and EPO metabolism in the liver was prolonged due to NPs [259]. There is currently no effective therapy that is available clinically for PVL and white matter injury in preterm infants and this is an area where there is significant urgency for novel therapies to be explored.

6.2.3. Nanoparticles evaluated in perinatal stroke

Although nanoparticle-mediated drug delivery has been extensively evaluated in adult experimental stroke models [260–269], there are no studies in animal models of perinatal stroke. Nevertheless, recently a study has been published where the effects of retinoic acid-loaded polymeric NPs in the prevention of ischemic injury in the immature brain were evaluated in vitro [270]. Retinoic acid-loaded polymeric NPs were found to inhibit cell death and normalize markers of neurovascular function and inflammation in hippocampal organotypic slice cultures exposed to oxygen and glucose deprivation.

Liposomes have been evaluated as drug delivery platforms in neonatal stroke. Although a detailed review of liposomes is beyond the scope of this article, these studies give us valuable information about therapeutic agents that may be neuroprotective in neonatal brain injury, if delivered to the brain effectively. Antisense *in vivo* knockdown of synaptotagmin I by hemagglutinating virus of Japan-liposome meditated gene transfer attenuates ischemic brain damage in neonates rats

Table 2
Lists the nanoparticle-drug conjugates in literature that have demonstrated efficacy in models of neonatal brain injury (abbreviations: hypoxic-ischemic encephalopathy=HIE, dendrimer=D, nanoparticle=NP, poly(amidoamine)=PAMAM, intraperitoneal=IP, intravenous=IV, subcutaneous=SQ, orally=O, postnatal day=P).

Disease model	HIE rat model (mild injury), right common carotid artery, recovery 1 h, hypoxia: 8% O2 for 75 mins	HIE rat model (moderate-severe injury), left common artery, recovery of at least 30 mins, hypoxia: 8% O2 for 135 mins	HIE piglet model (severe injury), only hypoxia or transient bilateral carotid ligation (15 min before and during hypoxia), and 40 min of hypoxia	Rabbit model of cerebral palsy, induced by maternal intrauterine endotoxin administration	Necrotizing enterocolitis (NEC) associated brain injury, brief hypoxia (5% O2 for 10 min twice daily) for 4 days; plus NEC induction using bacteria isolated from an infant with NEC	Ischemia-induced neonatal white matter injury, unilateral carotid artery ligation while kept mildly hypoxic for 15 min	Periventricular leukomalacia rat model induced by the administration of toxin 3-nitroproprionic acid (3-NP)	Periventricular leukomalacia rat model induced by the administration of toxin 3-nitroproprionic acid (3-NP)
Nanoparticle platform	PLGA-EPO-NP	PLGA-PEG/curcumin	Lecithin/cholesterol/2HBCD nanosomes quercetin	D-NAC	D-NAC	D-NAC	EPO-NP	EPO-NP
Antioxidant/ Therapeutic agent	Recombinant Erythropoietin (r-EPO)	Curcumin	Quercetin	N-Acetyl-l-Cysteine (NAC)	N-Acetyl-l-Cysteine (NAC)	N-Acetyl-l-Cysteine (NAC)	Erythropoietin (EPO)	Erythropoietin (EPO)
Polymer type	Poly-DL-lactide-coglycolide (PLGA)	Poly (lactic-co-glycolic acid)-poly(ethylene glycol) (PLGA-PEG)	lecithin/cholesterol/2-hydroxypropyl-beta-cyclodextrin (2HBCD)	generation-4 hydroxyl (G4-OH) PAMAM	generation-4 hydroxyl (G4-OH) PAMAM	generation-4 hydroxyl (G4-OH) PAMAM	Oligochitosan/sodium tripolyphosphate	Oligochitosan/sodium tripolyphosphate
Administration route	IP	IP	IV	IV	O	IP	IP	SQ
Concentration	30 U/kg, 100 U/kg, 300 U/kg	10 mg/kg	10 mg/kg	D-NAC with 1 mg/kg NAC or 10 mg/kg NAC	100 mg/kg	10 mg/kg	*n*=50 IU/kg	50 IU/kg
Administration time	1 h after HI and during 2 24-hours intervals	30 min, 24h and 48h after HI	during the reanimation period for 60 min or administered during 60 min as an infusion before ischemia/hypoxia	6 hours after birth (day 1)	48 h of starting NEC model, once a day for 2 days after	- at P6 (sub-acute), or - P10 (delayed)	1 h after on P5	on P5
Animal type	Sprague Dawley rat	Sprague Dawley rat	Piglet	Rabbit kit	C57Bl/6 mouse	CD-1 mouse	Rat	Sprague Dawley rat
Animal age	P10, full term	P7, preterm	Full term, up to 48 h from birth	on gestational day 28 (G28)	P7, preterm	P5, preterm	P5, preterm	P5, preterm
Adjacent therapy or comparative therapy	- 30 U/kg r-EPO - 100 U/kg r-EPO - 300 U/kg r-EPO - 5000 U/kg r-EPO - Sham	-Saline -Free curcumin 100 mg/kg -Blank PGLA-PEG	-Sham lesioned -Hypoxia -Nanosomes without quercetin	- PBS (positive control) - 10 mg/kg NAC - 100 mg/kg NAC - Dendrimer alone	-Saline	- 55 mg/kg of D-Cy5 at P6 - 55 mg/kg of D-Cy5 at P10	- Injured group - injured plus free EPO (n=5000 IU/kg) 1 h after 3-NP injection -Control	- Injured group - Control
Main results	- EPO-NP was 10-times more effective than regular r-EPO in neuroprotection - EPO-NP reduced infarction volume - EPO-NP improved long-term motor activity	-PLGA-PEG/curcumin reduced global injury, mostly in less severely injured rats -PLGA-PEG could cross the impaired BBB, extravasate into the brain parenchyma of the HI rats and localized in regions of injury -PLGA-PEG/curcumin reduced microglia	-Quercetin in lecithin/cholesterol/2HBCD nanosomes stabilized blood pressure after severe hypoxic episode, decreasing the need for oxygen supplementation -Recovery of spontaneous breathing, suckling and walking capacity	- D-NAC improved motor function - D-NAC suppressed markers of oxidative injury and inflammation - D-NAC suppressed pro-inflammatory microglia - D-NAC improved myelination and attenuated neuronal injury	-D-NAC reversed NEC-induced brain injury and prevented the neurocognitive impairments -D-NAC reduced ROS accumulation and microglial activation -D-NAC prevented the decrease of myelin basic protein expression and the loss of oligodendrocyte progenitor cells	- D-Cy5 uptake was both time- and injured region-dependent. - D-Cy5 localization was first in astrocytes and later in microglia - D-NAC, at either sub-acute or delayed time points after injury, suppress the pro-inflammation and decrease microglial activation, improving myelination	- 50 IU/kg EPO-NP had the same effect as a 5000 IU/kg direct injection of free EPO - EPO-NPs can attenuate liquefaction - EPO-NPs improve memory and agility - NPs prolonged the time course of EPO metabolization in the liver	-EPO-NPs improved general brain damage -EPO-NPs protected immature oligodendrocyte and preserved myelin basic protein -EPO-NPs significantly attenuated lipid peroxidation and nitrite concentration and restored superoxide dismutase and catalase enzyme activities
Reference	Chen et al., 2012	Joseph et al., 2018	Blasina et al., 2015	Kannan et al., 2012	Niño et al., 2018	Nance et al., 2015	Wang et al., 2012	He et al., 2010

[271]. The use of nanotherapies in perinatal stroke is understudied and is an area that should be explored further especially since there are no clinical therapies that are currently available for this population.

6.2.4. Nanotherapies for brain injury due to intrauterine or postnatal infection/inflammation

Since neuroinflammation mediated by activated microglia and astrocytes is known to play a key role in the pathogenesis of perinatal and neonatal brain injury, targeting the innate immune system in the brain has been a therapeutic strategy explored in several studies. Kannan and collaborators were one of the first groups to develop a nanotherapeutic approach for perinatal/neonatal brain injury. Hydroxyl terminated PAMAM dendrimers upon systemic administration were found to localize specifically in 'activated' microglia and astrocytes in newborn rabbits exposed to intrauterine inflammation, but not in normal, healthy, age-matched controls [235,272,273]. The extent of uptake in the brain correlated with the severity of the injury [273]. They demonstrated that systemic delivery of D-NAC was safe in the immature brain [235]. D-NAC treatment resulted in the suppression of neuroinflammation and in a significant improvement in motor function in rabbit kits with maternal inflammation induced cerebral palsy [235]. This was associated with improvement in inflammation, oxidative injury, myelination and neuronal cell counts. More recently, D-NAC was also shown to be effective in targeting activated microglia and improving long-term myelination up to adulthood in a mouse model of necrotizing enterocolitis induced brain injury. This was also associated with long term behavioral and cognitive improvement [237].

One of the other important points highlighted in these studies is that D-NAC was effective even when administered days after the insult. Dendrimer was found to localize in activated microglia even at postnatal day nine, or 12 days after the insult [273]. This indicates that the therapeutic window for treatment may be broader for perinatal brain injury than previously recognized. Besides NAC, the antibiotic minocycline (mino) conjugated to the dendrimer (D-mino) was tested in an *in vitro* model of activated murine microglial cells [274]. D-mino suppressed TNF-α production, and reduced oxidative stress by suppressing nitric oxide production *in vitro*, and intravenously administered Cy5—D-mino co-localized with activated microglia in the periventricular white matter areas *in vivo* in the rabbit model of neonatal neuroinflammation.

Although limited, the number of studies focusing on neonatal brain injury using nanotechnology approaches to enhance repair and regeneration is steadily increasing. Most of the work in this indication has been on the delivery of small molecule drugs and proteins/peptides. Gene therapy using nanotechnology has not been explored adequately in the context of neonatal brain injury. This is another area where NP delivery may be promising and has great potential for repair and regeneration following an injury. Although concerns for toxicity are always greater in the developing organism, the nanoparticle platforms that are currently being evaluated for neonatal brain injury appear to be generally safe and non-toxic. However, long term toxicology studies up to reproductive age, to evaluate effects on growth and maturation as per standard regulatory guidelines will be necessary for each of the nanoparticle-based therapies prior to clinical translation.

7. Conclusion

Major advances made in obstetric care and in neonatal intensive care in the last several decades have greatly reduced infant mortality and the mortality and morbidity associated with prematurity. However, despite the advances, the incidence of brain injury in neonates has not decreased substantially. This may be because of greater survival of more premature babies for whom the neurological morbidities still remain. Neurologic morbidities can range from severe motor and cognitive deficits to subtle behavioral and learning problems that can manifest later in life. These long term sequelae are often uniquely associated with injury to the developing brain. Since the brain at this age is rapidly growing and changing, response to an injury is also variable based on the gestational age and stage of maturation of different areas of the brain. Similarly, response to a therapy in the immature brain may be very different from that of an adult brain. Apart from standard considerations such as pharmacokinetics, metabolism and elimination that can be different in the neonate when compared to adults, physiologic differences should also be considered when designing novel therapies especially for an intensely dynamic system such as the developing brain. CNS repair is not simply a matter of cell replacement but also entails restoring the tissue architecture of the neural networks so that the newly generated cells can integrate into the circuitry. This may require the removal of injurious causal factors, modulation of inflammatory responses, protection from further degeneration and re-establishment of network connections across the lesioned sites.

There is greater recognition now that biological events that occur in utero or in the neonatal period can influence diseases in adulthood. Children are considered 'therapeutic orphans' because they often miss out on therapeutic advances while being exposed to avoidable risks. The implementation of the Pediatric Research Equity Act (PREA) and the Best Pharmaceuticals for Children Act (BPCA) have provided extension of market exclusivity incentives for studies that involve children. Although this has led to significant progress in therapeutic advances in children, it has unfortunately not translated to the same extent in neonates and there has been a relative neglect of this population [275]. This may be due to the greater risk-averseness regarding inclusion of neonates. However, when one considers that environmental factors that influence fetal and early postnatal brain development have been implicated in many pediatric and adult neurological disorders such as autism spectrum disorders, schizophrenia and other mental illnesses [276], it is clear that the socio-economic and personal burden is extremely high.

7.1. Future perspectives – nanothechnology for neuroregeneration in the injured newborn brain

There is an urgent need to design therapies that are specific to this age group where neuroregeneration can potentially have a very significant long term impact. Ultimately, an ideal therapeutic strategy could be nanotechnology that would target both suppression of injurious cascades and promotion of regeneration and repair. As detailed in this review, several pathophysiological processes such as oxidative stress, inflammation, and excitotoxicity, are involved in the unique and selective vulnerability of cells and brain regions in the dynamically developing perinatal brain. Thus, nanotherapies that target multiple pathways seem to be the key to achieving maximum benefit. When such therapies are combined with approaches that aid regeneration of the impaired brain tissue like the delivery of growth factors and stem cells, it may be possible to achieve normal brain development and functioning. The combination of a multipronged approach that can attenuate several pathways involved in injury along with the administration of stem cells and/or growth factors may allow for carefully planned, individually tailored treatments according to the gestational age and type of injury, taking us another step towards the goal of personalized medicine and healthcare.

Declaration of Competing Interest

None

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