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Concise Review

Stem Cell Therapy for Neuroprotection in the Growth-Restricted Newborn

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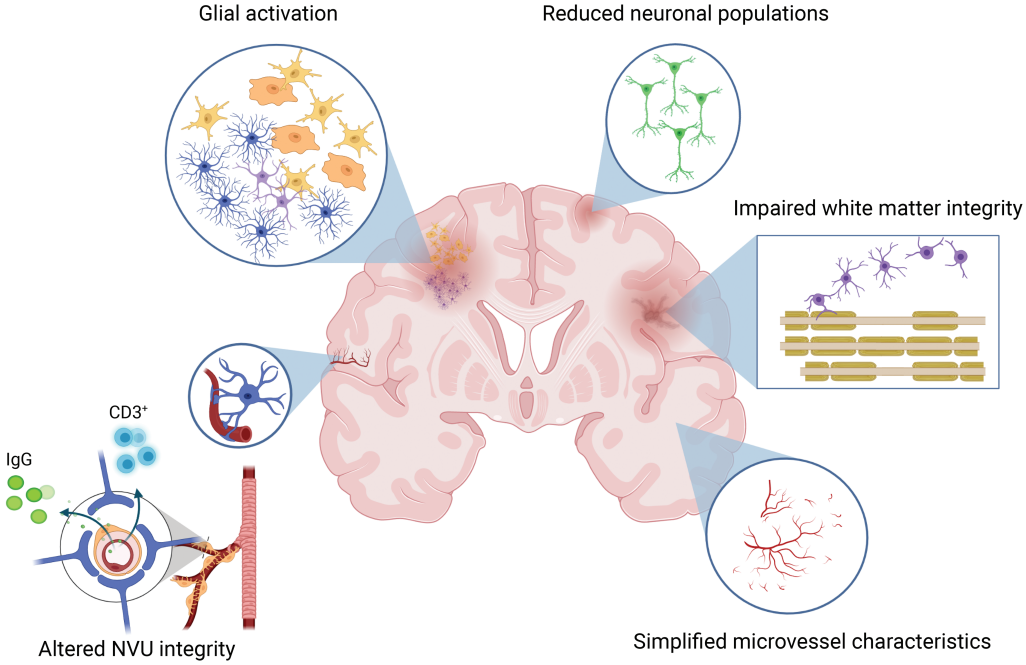
Abstract

Fetal growth restriction (FGR) occurs when a fetus is unable to grow normally due to inadequate nutrient and oxygen supply from the placenta. Children born with FGR are at high risk of lifelong adverse neurodevelopmental outcomes, such as cerebral palsy, behavioral issues, and learning and attention difficulties. Unfortunately, there is no treatment to protect the FGR newborn from these adverse neurological outcomes. Chronic inflammation and vascular disruption are prevalent in the brains of FGR neonates and therefore targeted treatments may be key to neuroprotection. Tissue repair and regeneration via stem cell therapies have emerged as a potential clinical intervention for FGR babies at risk for neurological impairment and long-term disability. This review discusses the advancement of research into stem cell therapy for treating neurological diseases and how this may be extended for use in the FGR newborn. Leading preclinical studies using stem cell therapies in FGR animal models will be highlighted and the near-term steps that need to be taken for the development of future clinical trials.

Key words: newborn brain; fetal growth retardation; stem cells; mesenchymal stromal cells; endothelial progenitor cells.

Graphical Abstract



Fetal growth restriction (FGR) results in significant neurodevelopmental issues via chronic ischemia that can lead to lifelong physical and mental disabilities. This review highlights the latest research in the development of stem cell therapies in preclinical and clinical trials aiming at repairing and reversing the neuronal damage, as well as discussing the gaps that remain in this field of research.

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Significance Statement

There are currently no clinical treatments available to reverse the long-term neuronal damage that is observed in fetal growth-restricted newborns. Lifelong physical and learning disabilities are challenges these children will face and therefore the need to rapidly develop stem cell therapies for neuronal repair and regeneration is immensely required. This concise review covers the latest seminal preclinical and clinical studies in the field and provides important commentary on the development of stem cell therapies for treating neuronal injury in fetal growth-restricted newborns.

Introduction

Fetal growth restriction (FGR), the failure of a fetus to achieve normal growth potential, is a leading cause of perinatal morbidity and mortality.[1-4] The incidence frequency of this condition is at its highest rate in the last 20 years, accounting for ~10% of all pregnancies in both developing and industrialized nations.[5-7] A study of 138 low- and middle-income countries found of the 135 million births investigated, 29.7 million babies born at term possessed FGR indications.[8] The causes of FGR are complex with manifestations of pathophysiological processes arising from several potential sources, including maternal (hypertension, preeclampsia, malnutrition), placental (placental dysfunction leading to oxidative stress), fetal (multiple gestation, chromosomal abnormalities), and genetics.[9] Compromised placental function can result in chronic fetal hypoxia and inadequate oxygen supply or aberrant transfer of maternal hormones to fetal circulation.[10,11] These have important implications on fetal programming, growth, and development.[4]

FGR can be broadly classified into 2 groups, early onset, occurring before 32-week post-conceptional age (symmetric FGR) or late onset, occurring during the third trimester (asymmetric FGR). Fetal circulatory redistribution is associated with late-onset FGR and is the result of preferential redistribution of combined ventricular output to the brain and heart compared with peripheral organs. This "brain-sparing" effect, termed asymmetrical FGR, is the most common form of growth restriction, affecting 70%-80% of all FGR infants.[9] Early onset or symmetrical FGR is characterized by global growth restriction throughout pregnancy and accounts for 20%-25% of FGR fetuses. Brain-sparing is considered a protective mechanism in the FGR condition, yet it incompletely protects the brain from adverse neural outcomes associated with FGR. Recent evidence, however, has questioned this theory, with several studies demonstrating that asymmetric FGR infants have a worse neurodevelopmental outcome than their symmetric FGR neonate counterparts.[12-18] Nonetheless, while brain injury severity is variable among FGR newborns, there remains a critical need for neuroprotection in these infants.

As a result of FGR, these children are often at a greater risk of developing lifelong adverse health impacts. This includes neurodevelopmental outcomes, such as cerebral palsy, behavioral issues, and learning and attention difficulties, with neurodevelopmental disabilities reported in 24%-53% of FGR infants at 2 years of age.[19,20] These adverse outcomes can also extend into cardiovascular complications, diabetes, and hypertension.[21-24] Many of these FGR neurological outcomes have been attributed to impaired vascular development, resulting in chronic inflammation, and disrupting central nervous system (CNS) development during gestation.[4,25,26] As clinical management of pregnancies associated with FGR fetuses improves, overall medical care has resulted in an increased survival rate for FGR newborns. However, accompanying improved rates of survival is a greater burden of disability, long-term medical care, and a general increase in necessary support associated with FGR. The scope of treatment for these neurological diseases is largely limited, yet over the last decade, stem cell therapies have generated interest due to their potential in treating and reversing neurological disorders.[27,28] Stem/progenitor cells exhibit unique functional characteristics, making them attractive for future clinical therapies, such as by enhancing differentiation and proliferation capacity around damaged tissues thus driving repair, and by exerting potent paracrine immunomodulatory effects, potentially diffusing chronic inflammation.[29-31] Recent studies have shown that transplanted stem cells have an innate ability to migrate to damaged areas and assume the function of neurons in models of Parkinson's disease, Alzheimer's disease (AD), spinal cord injury, cerebral palsy, and ischemic stroke.[31-33] Incredibly, greater than 230 clinical studies using stem cell approaches treating neurological disease have been registered (Clinicaltrials.gov). While to date no FGR neonate clinical trials with stem cell application have been actioned, preclinical trials are emerging. In this review, we will discuss current available clinical therapies for FGR newborns, and report on the development of preclinical tissue regenerative stem cell therapies for treating FGR.

Neurodevelopmental Deficits in FGR

Neurodevelopmental deficits are common in children having suffered FGR with structural anomalies reported in both fetal and newborn brains. Specifically, fetal studies have shown reduced brain volume and perturbed brain morphology, including decreased cortical folding.[34-36] In conjunction with this, FGR newborns display reduced head circumference compared with appropriately grown infants, which is a strong predictor of neurodevelopmental outcome.[37,38] Structurally, newborns with FGR are reported to have decreased cortical gray matter, perturbed cortical gyrification, and altered myelination of white matter compared with healthy newborns.[37,39,40] Imaging studies using MRI have demonstrated alterations in white matter development, organization, and connectivity in FGR infants at 12 months of age.[41,42] These early structural alterations are associated with deficits in long-term neurodevelopment. By 2 years of age, FGR infants demonstrate significantly lower motor and cognitive outcomes, which is maintained into school-age childhood when compared with age-matched preterm or term children.[43-45] These in-depth longitudinal studies of FGR infants demonstrate persistent neurodevelopmental deficits maintained into late childhood (6-10 years). This includes a higher incidence of lower cognitive performance, reduced memory, and visual-motor performance. Deficits in fine and gross motor function, memory, and increased hyperactivity are also noted in children born with FGR.[46-49] Due to these early and

persistent alterations in brain structure, along with associated neurodevelopmental deficits, there is a critical need for clinical intervention to treat the FGR newborn.

Current Clinical Interventions to Treat the FGR Brain

At present, there are no accepted therapeutic interventions for the FGR fetus other than modified neonate delivery. While treating in utero may afford neuroprotection, up to 50% of FGR babies are diagnosed in their late-gestation period, or even at birth.[50-52] Although it is important to note, this excludes cases of early-onset FGR which consistently present before 28-week gestation. The push for strategies to reduce brain deficits in FGR newborns grows as FGR burden increases, however, as of yet, very few trials have been undertaken. Prevention of FGR is a common target but is a challenging prospect with most studies instead of focusing on maternal pharmacological interventions to improve placental to fetal perfusion during pregnancy.

Antenatal Pharmacological Interventions for FGR

A small set of clinical trials have focused on preserving the growth of the FGR fetuses using maternal supplementation, such as sildenafil (trial canceled due to neonatal deaths) and arginine.[53-56] An even more limited number of clinical trials have aimed to directly protect the FGR fetus/newborn brain. The EVERREST Project (NCT02097667; active, not recruiting) aims to deliver vascular endothelial growth factor (VEGF) via an adenoviral vector to the uterine artery to aid in increased blood flow and fetal growth but has yet to report neurodevelopmental outcomes. Melatonin (NCT01695070; completed) and allopurinol (NCT00346463; not yet recruiting) are other maternal treatments with neurodevelopmental outcomes pending. One study that has reported neonatal brain outcomes following antenatal treatment was maternal administration of polyphenol-rich pomegranate juice in mothers carrying FGR pregnancies (NCT00788866). While this reported no differences in brain injury, metrics or volume, altered white matter organization, and functional connectivity were observed in the treated group warranting further studies into this treatment.[57] However, while this study appears promising, the results were not definitive, with variability in timing of detection and delivery likely contributing to the lack of beneficial effects observed in clinical outcomes following maternal interventional studies.

Postnatal Physiological Interventions for FGR

Due to the lack of effective antenatal treatments available, the most viable option in severe cases of FGR is preterm delivery, which itself is associated with inherent risk factors.[58] Preterm birth is associated with increased risks of significant comorbidities, including respiratory, cardiometabolic, neurosensory impairment, and neurodevelopmental disorders that can manifest throughout life.[59-61] FGR preterm infants are at the highest risk for long-term neurodevelopmental disabilities, such as cerebral palsy, mental retardation, and learning and behavioral issues.[62-66] This preterm FGR group may largely represent early onset FGR. It is notable to reiterate the different groups of FGR (early vs late onset) have likely different pathologies and timing of presentation and such, when intervention is considered. Yet, treating ex utero, as close to birth as possible, may enable better therapeutic outcomes, and will optimize treatments to target both early- and late-onset FGR neonates. However, surprisingly, there are currently no clinical trials targeting FGR newborns with the goal of alleviating neurodevelopmental issues. Further studies are required to address this fundamental gap in neonatology, to protect these vulnerable babies from lifelong disorders.

Targeting Inflammation and the Neurovascular Unit to Protect the FGR Brain

To enable these future studies, it is important to identify therapeutically targetable mechanisms of brain injury in the FGR newborn. Recent animal investigations have revealed multiple mechanisms, which mediate cellular injury in the brains of FGR neonates (Fig. 1). These include excitotoxicity, oxidative stress, necrotic and apoptotic degeneration, neuroinflammation, and blood-brain barrier (BBB) disruption.[67-71] Neuroinflammation is emerging as one of the key mechanisms mediating abnormal brain development in FGR, encompassing a number of processes, including increased microglia numbers, astrogliosis, elevated production of pro-inflammatory cytokines, decreased production of anti-inflammatory cytokines, release of chemokines, and infiltration of leukocytes.[26] A recent human study demonstrates evidence of an inflammatory event correlating with abnormal neurological outcomes in FGR infants. FGR neonates present with elevated pro-inflammatory cytokines in the blood at 2 weeks of age, a finding which correlates with adverse neurodevelopmental outcomes at 2 years of age.[72,73] Recent studies have also demonstrated the role of inflammation in FGR using experimental models, including rodents, sheep, and porcine.[26] A pro-inflammatory state is evident in the brains of FGR neonates at postnatal day 1, which persists until at least day 4 in a preclinical pig model of spontaneous FGR.[74] This pro-inflammatory state is detrimental to neuronal and white matter development in FGR brains.[26] Animal studies have shown where inflammation is prevalent in certain brain regions, such as the parietal cortex, both mature and immature neuronal and oligodendrocyte populations are negatively impacted in the brains of FGR neonates with cellular disruption and loss and an increase in pro-inflammatory cytokine expression on neurons.[74,75] Further studies demonstrate treatments, such as ibuprofen and melatonin, that target inflammatory pathways attenuate neuropathology associated with FGR in large animal models, however, long-term safety and efficacy studies are required.[75-77] These studies highlight the potential for targeting inflammation following birth, leading to reduced or abolished ongoing neurodevelopmental issues in FGR newborns.

Inflammation may be a key mediator contributing to neurovascular unit (NVU) dysfunction in neuropathological conditions.[78] The NVU is a multicellular compartment of the CNS, which acts as a barrier separating the brain from the blood (BBB). Cells that comprise the NVU include vascular endothelial cells, glial cells (astrocytes and microglia), neurons, pericytes, and the basement membrane. The NVU plays a critical role in protecting the brain against the entry of toxic substances, which can have long-term pathological effects on the brain. Recent studies in large animal models of FGR have demonstrated NVU disruption with concurrent inflammatory response and immune cell infiltration into the FGR brain.[76,79] In the brains of FGR neonates, a reduction in the number of endothelial cells is evident with a loss of interaction between astrocytic end-feet and blood vessels. In addition to activated microglia in the perivascular space, plasma protein leakage is evident, suggestive of structural

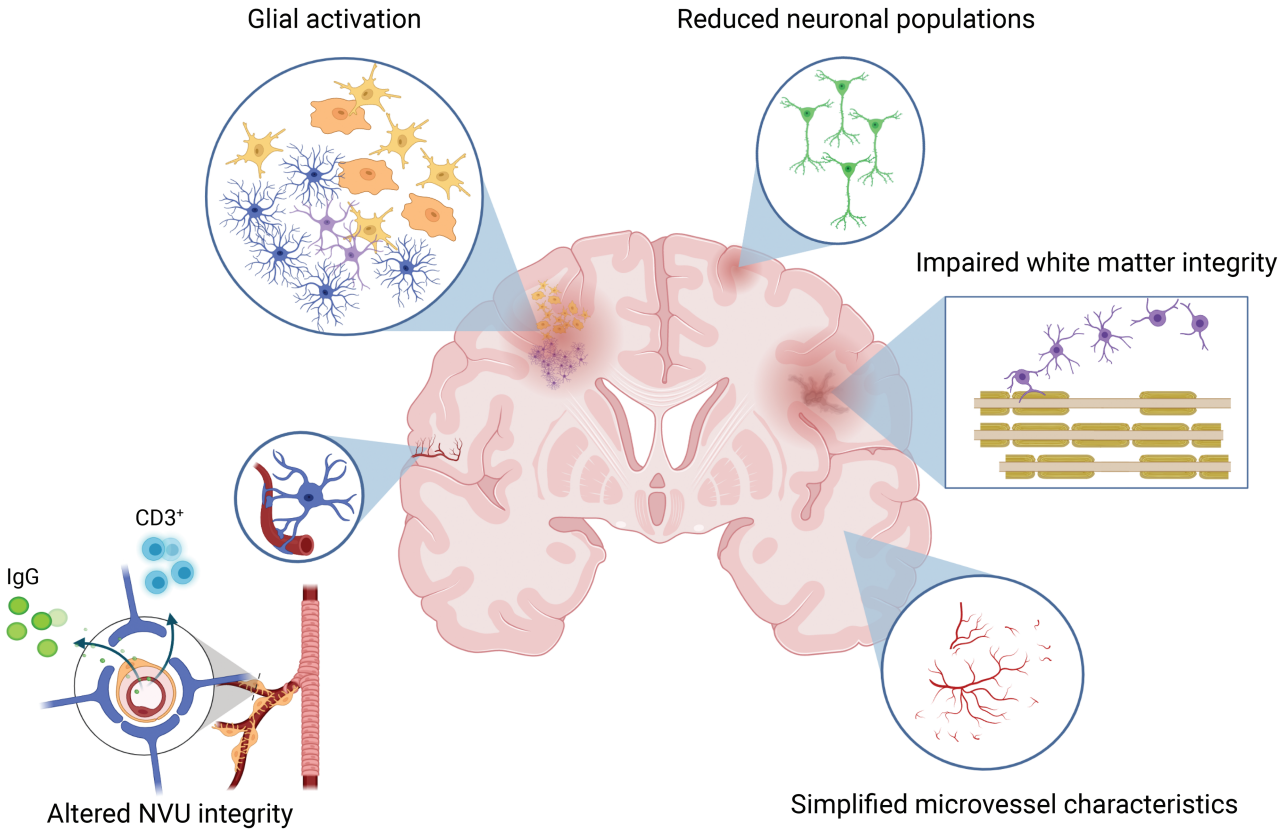

Figure 1. Potential structural and cellular changes associated with the neuropathology of fetal growth restriction (FGR) newborn. Chronic exposure to reduced oxygen and nutrient supply elicits an ongoing pro-inflammatory environment for the developing brain. Microglia and astrocytes display the maladaptive inflammatory response, with activated/reactive morphology and the release of pro-inflammatory cytokines, such as interleukin-1β, TNF, and CXCL10, further exacerbating the inflammatory response. Altered interaction of astrocytes with microvessels may be associated with the influx of peripheral infiltrates, such as serum proteins (IgG) and T cells (CD^{3+}). There is also evidence of altered vasculature which may worsen oxygen and nutrient supply to cellular bodies during postnatal development hindering maturation and repair. Impaired myelination of white matter regions is associated with decreased oligodendrocyte expression. FGR newborn brains displayed reduced numbers of mature neuronal cells throughout the somatosensory cortex. Altered neuronal populations and connectivity, due to disrupted white matter tracts, may be associated with deficits with the integration of information from different modalities. Illustration created with Biorender.com.

deficits to the NVU.[76] Treatment with anti-inflammatory drugs (ibuprofen, melatonin) not only lessened the inflammatory response but also resulted in reduced NVU disruption with normalized glial vessel interaction indicative of a healthy brain microenvironment.[76,77] These studies demonstrate NVU disruption in FGR may exacerbate early neuroinflammatory responses and therefore early targeting of both inflammatory pathways and NVU may provide a therapeutic approach in protecting the FGR newborn.

Stem Cells in Treating Neurological Disease

Biological understanding of stem cell populations that exist within the body has increased significantly in the past 2 decades.[80,81] During this time, several stem cell populations have been identified in the context of treating neurological diseases, such as those derived from the programming of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). This includes neural stem cells (NSCs). Mesenchymal stromal cells (MSCs), amniotic epithelial cells (AECs), and endothelial progenitor cells (EPCs) have also been studied in neurological disease treatment.[29,31,82] These populations have been successfully isolated from fetal (placenta, umbilical cord blood [UCB]) and adult (adipose, dental pulp, bone marrow, brain) tissues.[83-87] Additionally, the advent and development of iPSCs by Yamanaka et al, set the stage for stem cells as a proxy for investigation into the genetic basis of both neuronal homeostasis and neurological disease development, while also generating interest in the potential large-scale application of stem cells in clinical tissue repair and regeneration therapy.[88] The preclinical and clinical application of these stem cell treatments have, thus far, aimed to repair neurological damage by repopulating astrocytes and oligodendrocytes using NSCs, restoring blood flow with EPCs by driving vasculogenesis/angiogenesis, and defusing chronic inflammation through the potent immunomodulatory and paracrine effects of MSCs and AECs.[29,31,33]

Specifically, NSCs derived from embryonic origins have been successfully transplanted in preclinical models of multiple sclerosis (MS) and stroke, whereby reconstitution of lost oligodendrocyte myelin sheaths following stem cell treatment attenuated disease progression.[89] Importantly, potent neurotrophic effects were observed through the production of various immunomodulatory cytokines, driving regeneration and repairing not only neuronal damage but also vascular structures, enabling blood flow restoration to ischemic regions.[90,91] However, a major downfall of NSCs is their inability for long-term in vivo survival and engraftment. Moreover, difficulty in obtaining clinically relevant quantities for large-scale patient delivery remains a major hurdle in the

field.[92] NSCs, astrocytes, and microglia derived through iPSCs reprogramming have been powerful in genetic characterization and pathogenetic studies of neurological disease in in vitro modeling in neurological diseases, such as AD and may offer a solution to obtaining clinically relevant quantities of NSCs for treatment.[93] However, there have been challenges in translating iPSC technology from in vitro to in vivo tissue regeneration treatments. For example, defining the right conditions for neuronal iPSC in vivo transplantation has yet been completely resolved, while the prevention of iPSC tumorigenicity in the host remains to be fully addressed.[94] It should be noted that there is also ongoing research into in vivo cellular reprogramming, such as in the case of astrocytes to functional neurons to lessen the burden of both astrocyte activation and neuronal loss in injury.[95]

MSC cell therapy may prove to be more effective in a clinical setting than iPSC-derived NSCs as they are readily isolated and expanded, making them one of the most rapidly developing areas of neurological regenerative medicine.[96] This ease of isolation and expansion from various tissue sources, coupled with their low immunogenicity and immunomodulatory abilities enables their use in allogeneic clinical trials and confers feasibility in terms of donor application.[97,98] Additionally, MSCs possess strong antiapoptotic, paracrine, and multidirectional differentiation capacity, and therefore have been trialed in numerous neurological disorders, such as AD,[99,100] amyotrophic lateral sclerosis,[101] Huntington's disease,[102,103] MS,[104] and spinal cord injury,[105] with many of these conditions having little to no effective clinical treatment currently available.[96] To date, MSCs have been most effective when used in models of stroke. Following MSC infusion, reductions in brain inflammation, edema, and lesional areas were identified in different animal stroke models.[106-108] This was correlated with improved functional recovery and promotion of axonal growth and neurogenesis.[109,110] Stroke, as an ischemic event, not only affects neurons but also other cell populations, particularly endothelial cells which are squamous cells that line vasculature. MSCs homing to ischemic regions in the brain have been shown to stimulate angiogenesis and vasculogenesis, driven through potent paracrine activity through factors, such as VEGF.[109,111,112] Additionally, MSCs have also been shown to reduce leakage and permeability of brain vasculature, with recent studies showing interaction with pericytes, astrocytes, and neurons, as well as providing BBB integrity and maintenance.[96] Preclinical trials of hypoxic-ischemic encephalopathic (HIE) have also used extracellular vesicles isolated from MSC populations, with results demonstrating significantly reduced brain inflammation and tissue apoptosis, highlighting the powerful paracrine activity of MSC (reviewed extensively by Matei et al[113]).

EPCs are another stem cell population of significant interest in treating stroke-induced ischemic damage.[114] EPCs are known to promote and drive angiogenesis via growth factors (eg, VEGF) and cytokines (interleukin-6 and -8) via paracrine activity, however, their major advantage in neurological disease treatment is differentiation, giving rise to mature functional endothelial cells, repairing damaged endothelium, and restoring vascular function.[82,115-118] Recent studies have shown that the transfusion of EPCs results in significant blood flow improvement to ischemic regions, but more importantly, demonstrates active vasculogenesis through chimeric vascular network integration within the host cardiovascular system.[119] Thus, EPC engraftment and survival, enabling vascular recovery, may be an essential component of clinical stem cell therapy for neurological disorders, such as FGR in the future.

Lastly, it must be noted that there is always the concern for immunogenic rejection and/or graft vs host disease when delivering a transplantation of allogeneic or xenogeneic donor material as in the case with stem cell therapies. Whether in preclinical or clinical trials, a close assessment of health and safety parameters are essential to ensuring adverse events are avoided or prevented but more importantly are readily reported to provide guidance for future trials, particularly with dosing regimens and cohort groupings.

Stem Cell Delivery in Clinical Trials of FGR

There are currently no clinical trials that examine the neuroprotective potential of stem cell treatment for FGR newborns. However, there are several trials currently registered pertaining to adjacent neonatal neurodevelopmental and cardiovascular diseases, such as HIE and preterm neonates. For example, a pilot study of 5 HIE newborns reported no significant adverse effects following autologous UCB cell delivery, with the survival of infants up to 1 year.[120] Furthermore, promising results from a phase I clinical trial of autologous UCB cells in 23 HIE neonates (NCT00593242) have shown increased survival and improved neurodevelopmental outcomes up to 1 year of age.[121] While the results from these 2 studies are significant for neonatal stem cell disease clinical treatment, safety and efficacy data from larger multicenter clinical trials are yet to be reported. Moreover, HIE is generally an acute onset condition occurring at birth, which is in contrast with chronic brain remodeling seen in FGR infants pre and post-birth.

Stem Cell Delivery in Preclinical Trials of FGR

In addition to clinical trials of FGR infants lacking, preclinical animal model research is also limited, with only 3 published studies to date (Table 1). These studies, including an FGR rat model and 2 larger animal investigations, show promising neuroprotective results, reporting multi-compartmental benefits following stem cell treatment of the brain.[122-124]

As discussed earlier, disruption of the NVU is present within the brains of FGR neonates and results in an influx of detrimental immunomodulatory substances, such as pro-inflammatory cytokines. Stability of the NVU in the developing brain is critical for a healthy brain environment. One study in a clinically relevant model of FGR, in which asymmetric growth restriction occurs spontaneously in runted piglets,[125] examined combination treatment of 2 stem cell populations on the NVU in the FGR piglet brain. The authors examined the effects of dual human fetal MSC and endothelial colony-forming cells (ECFC—an EPC) treatment, both isolated from the human placenta, in neonatal FGR piglets.[124] This combination treatment (termed cECFC) resulted in ECFC priming, increasing their homing and engraftment capacity to damage vascular tissue in the brain. Following cECFC treatment, not only was an increase in vessel density evident, but also an improvement in vascular length without vessel branching. This suggests that eECFC treatment improves brain vasculature without promoting excessive angiogenesis.[124] The same study showed BBB integrity was also improved following cECFC treatment in the FGR piglet with reduced albumin leakage into the brain.[124] Conversely, MSCs delivered alone did not exert a significant effect on improving the vasculature in the brains of FGR

Table 1. Summary of preclinical trials for FGR stem cell delivery.

Study	Model	Induction of FGR	Cell type	Harvesting	Age	Administration route	Treatment outcomes
Kitase et al, 2020	Rat	Constriction of uterine arteries at 17-day gestation (human equivalent 20-25 weeks)	Umbilical cord MSC (UC-MSC)	Human umbilical cord. Passage 4 was used	*Administration:* Postnatal day 4 *Brain equivalence:* Human 32- to 34-week gestation *Brain endpoint:* 2 months (human young adults)	1×10^5 UC-MSC i.v. via jugular vein	- Mortality rate: 20.8% vehicle and 15.4% - Improved behavioral outcomes (negative geotaxis at P11 and rotarod at 5 months) compared with vehicle - Increased neuronal cell count in the cortex at 2 months compared with vehicle - Increased Iba-1 cells, with elevated ED-1 (M1 polarized microglia) and CD206 (M2 polarized microglia) compared with sham - No change in astrocytes observed
Malhotra et al, 2020	Lamb	Single umbilical artery ligation at 88-day gestation	Umbilical cord blood stem cell (UCBC)	Healthy term lamb cord blood (144- to 145-day gestation). Isolated from buffy coat. Resuspended with FBS with 10% DMSO	*Administration:* Preterm; 126-day gestation *Brain equivalence:* 95 days = <24 weeks 135 days—term *Brain endpoint:* 24 hours (human preterm)	25×10^6 UCBC per kg i.v. via umbilical vein	- Decreased microglia in PVWM, SCWM, and SVZ - No significant difference in astrocyte cell count - Serum TNF-α levels lower compared with untreated FGR - No significant differences in oxidative stress (HNE) or cell death (caspase-3) - Increased cell proliferation in SVZ and increased Ki-67-positive blood vessels in the PVWM and SVZ - Increased GLUT-1 and pericytes at blood vessels - Decreased BBB dysfunction in FGR + UCBC (1/6 with albumin extravasation) compared with FGR (5/6)
Chand et al, 2021	Pig	Spontaneous FGR	Combined endothelial colony-forming cells (ECFCs) and mesenchymal stromal cells (MSCs)	Healthy human placenta. Isolated fetal ECFC and MSC were cultured	*Administration:* Postnatal day 1 *Brain equivalence:* Human term equivalent *Brain endpoint:* P4 (1-week postnatal in human)	1×10^6 ECFC and 1×10^6 MSC i.v. via mammary vein	- Improved expression of vascular markers (Col IV, CD31, and CD34) compared with FGR - Decreased BBB dysfunction, with less albumin and IgG staining compared with FGR - Decreased glial activation (Iba-1 and GFAP) in PC, IGWM, and PVWM compared with FGR - Enhanced anti-inflammatory cytokine expression (eg, IL-4, IL-10, and TGF-β2) compared with FGR - Normalized neuronal (NeuN and MAP2) and white matter integrity (MBP and Olig2) compared with normally grown brains

Abbreviations: BBB, blood-brain barrier; DMSO, dimethyl sulfoxide; ED-1, ectodysplasin A; FBS, fetal bovine serum; FGR, fetal growth restriction; GFAP, glial fibrillary acidic protein; GLUT-1, glucose transporter type-1; HNE, 4-Hydroxy-trans 2-nonenal; i.v.; intravenous; IGWM, intragyral white matter; MAP2, microtubule associated protein 2; MBP; myelin basic protein; PC, parietal cortex; PVWM, perivascular white matter; SCWM, subcortical white matter; SVZ, subventricular; TGF-β2, transforming growth factors-beta 2; TNF-α, tumour necrosis factor-alpha.

piglets 3 days after treatment. In addition to these findings, these human stem cell populations survived in immunocompetent piglets without the need for deleterious immunosuppressive therapy (eg, cyclosporine), emphasizing their potential for non-immunogenic allogeneic transplantation in infants. However, it is not yet clear whether these cells participate in off-target engraftment events, such as in the lungs and other organs. In support of these findings, an FGR lamb model having undergone allogeneic UCB mononuclear cells delivery 1-hour post-birth after was shown to support BBB integrity through reduced albumin extravasation into the brain, an endogenous serum protein that is not present in healthy brain tissue.[123] Impaired astrocyte end-feet interaction with blood vessels may also be associated with altered NVU integrity and BBB permeability in the brains of FGR neonates. Following cECFC treatment in the FGR piglet, astrocytes displayed a normalized glial vessel interaction similar to the normally grown piglets with consistent contact along the vasculature, suggesting glial cells conversion to a supportive role.[124] While UCB treatment did not alter astrogliosis in the FGR lamb, the authors observed an increased association of smooth muscle proteins of the basal lamina with pericytes in the NVU.[123]

Interestingly, the activation of microglia, key inflammatory cells in the brain, was found to be specifically modulated by MSC-only application. This was evident due to a decrease in both the number of microglia and their activation state throughout the brain parenchyma. However, this treatment did not significantly reduce the increased number of astrocytes present in the brain parenchyma of the FGR piglet.[124] These results are again corroborated by an FGR lamb study where UCB treatment reduced activated microglial cells but had no effect on astrocytes.[123] Furthermore, an FGR rat study also showed that umbilical cord-derived mesenchymal stromal cells (UC-MSC) did not affect the number of astrocytes 7 days after treatment.[122] cECFC treatment, however, may provide some respite from inflammatory cell activation in the brains of FGR neonates, with dual MSC and ECFC application minimizing both microglial and astrocyte activation in the FGR piglet brain.[124] This glial response in the brain tissue parenchyma may be due to restoration of cerebrovascularization and NVU integrity, attributed to the presence of ECFCs. It is, however, important to note that different developmental ontological stages were examined between the species. Specifically, while the brain growth spurt occurs post-natally in the rodent, it is evident prenatally in the sheep, while piglet brain growth trajectory is not dissimilar to the human newborn.[126]

Inflammatory cytokine response also plays a large role in progression of brain injury in the FGR newborn. In the FGR piglet, cECFC treatment had a greater effect on increasing anti-inflammatory cytokines rather than decreasing pro-inflammatory cytokines.[124] This prominent increase in anti-inflammatory cytokines may be due to reprogramming of the microglia into an anti-inflammatory state, as seen in an in vitro study.[127] Similar results have also been demonstrated in the FGR rat following UC-MSC treatment whereby treatment did not significantly affect pro-inflammatory microglial activation but simultaneously increased anti-inflammatory microglia, demonstrating a strong anti-inflammatory effect of stem cell treatment in the FGR rat.[122] In the FGR piglet, not only did cECFC treatment have a positive effect on the inflammatory response and NVU, but this treatment also reduced apoptotic activity as well as recovery to both gray and white matter cellular impairment.[124]

Behavioral and neurodevelopmental outcomes have not been thoroughly investigated using the preclinical animal models. Kitase et al used a negative geotaxis test to assess the maturity of vestibular receptors, central sensory function, and motor function and found significantly improved scores at P11 following UC-MSC treated FGR compared with the vehicle group.[122] They followed with rotarod testing at postnatal days 154-155 to assess balance and coordination, again finding significant improvement between UC-MSC-treated FGR compared with vehicle treatment. The findings of this study support the observed deficits in children born with FGR, such as altered coordination.[128]

At 12 months of age, infants with FGR displayed increased connectivity of the visual network and decreased connectivity of the auditory and language, and dorsal attention networks.[129] White matter injury observed in the FGR is likely influencing the connectivity of these networks. Periventricular white matter damage in the preterm brain following hypoxia/ischemia occurs during key transitional periods of oligodendrocyte lineage (pre-oligodendrocyte to immature to mature).[130] Tolsa et al demonstrated reduced total gray volume in the cerebral cortex of FGR brain which is associated with a decrease in neuronal cell populations.[37] Postmortem analysis of FGR brain found a reduction in a total number of cells at the cortical plate, indicating a decrease in potential future cell populations (estimated to be half that of a normally grown brain).[131] These findings demonstrate regional vulnerability with a range of cell types likely influenced, including neuronal and oligo-glial cells. Findings from the limited preclinical studies suggest positive benefits of stem cell treatments, including increased cell proliferation in the subventricular zone and improved white matter integrity determined by increased Olig2 and myelin basic protein.

Timing and Method of Stem Cell Delivery in FGR

While FGR is a chronic event, it is not globally ischemic, with differential distribution of regional cerebral blood flow occurring over time leading to adverse brain events beginning at 27-week gestation and persisting post-natally.[67,131] This is recapitulated in the FGR piglet where significant brain injury is first observed at 104 days, equivalent to 26- to 28-week human gestation.[132] This is a time of high NVU vulnerability due to cerebral vessel fragility,[133] but also high neurological plasticity with glial and NSC cell proliferation,[134] strong synaptogenesis, and accelerated myelination.[135] This suggests intrauterine therapeutic FGR intervention may provide the best chance of pathological amelioration and recovery. For example, intrauterine umbilical vein MSC delivery is currently being investigated for the treatment of congenital diseases.[136,137] However, as outlined above, there is limited capability for FGR preterm detection, suggesting that to cater to the current state of clinical capabilities, postnatal stem cell therapy remains the most accessible. This is not without benefit, however, as studies have demonstrated that alteration of growth processes in utero are post-natally amendable, improving FGR outcomes with much neuroplasticity maintained up to 2-year post-birth, including a prematurity of the NVU.[135,138] For example, MSC intranasal delivery was shown to improve sensorimotor and cognitive function in rodent

pups up to 10-day post-ischemic injury.[139,140] Regardless, spatial and temporal profiling of inflammatory events and NVU disruption in the FGR fetus, newborn, and infant would provide definitive information on optimal treatment timing.

An additional consideration in the development of clinical FGR treatment is the method of stem cell delivery. There are no studies that directly compare delivery methods for preclinical FGR or infant neurological disorders, with intravenous (IV) delivery currently considered the most favorable due to low invasiveness and effective neonatal FGR outcomes.[122-124] However, little investigation has been done into off-target homing and sequestering of systemic stem cell delivery in neonatal models. This is of concern as IV delivery is associated with peripheral organ pooling, such as in the lung, leading to reduced neurological efficacy and may be correlated with pulmonary complications.[141] Other methods of stem cell delivery include highly targeted intraparenchymal delivery, which has successfully treated murine neonatal hypoxic-ischemic brain injury using MSCs,[142] and intranasal delivery, which is minimally invasive and has gained popularity in recent years due to favorable neonatal rat HIE and stroke outcomes.[139,140,143] However, neither of these administration methods may be suitable for the emerging cECFC therapy due to bypassing of the BBB. To elucidate the most effective method of delivery, further research is required into stem cell peripheral organ homing and sequestering, as well as the mechanistic action and integration of stem cells in neonatal FGR treatment.

Conclusion

In summary, neurological impairment due to FGR is a chronic condition and causes significant long-term impairment, with little to no treatment currently available. As our understanding of the biology of stem cell populations increases, coupled with the development of encouraging and successful preclinical studies in regenerating neuronal and vascular structures and reducing inflammation, the advancement of a cell therapy to be used in FGR neonates soon after birth certainly warrants further investigation. From the evidence presented, combined MSC and ECFC therapy provides the greatest potential yet as a neuroprotectant for FGR and may even stimulate further research into multicellular approaches for other inflammatory and NVU neurological disorders.

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Conflict of Interest

The authors declared no potential conflicts of interest.

Author Contributions

K.C., R.N., J.W., and J.P. all contributed to manuscript writing. K.C. developed the figure. J.W. and J.P. provided final approval of manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

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