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REVIEW

Advancements in Neonatal Brain Injury Treatment: Nanomedicine-Based Strategies

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Abstract: Neonatal brain injury, such as hypoxic-ischemic encephalopathy (HIE), is a leading cause of infant mortality and long-term neurodevelopmental disabilities. Current clinical therapeutic strategies are limited by the blood-brain barrier (BBB), the complexity of the injury cascade, and the narrow therapeutic window. Nanomedicine has shown potential in preclinical studies for overcoming these barriers by leveraging its unique nanoscale characteristics and engineerability design to load, stabilize, and deliver vulnerable biomacromolecules across the compromised BBB to the lesion site. This review presents the first systematic horizontal comparison and critical evaluation of the major nanoplatforms employed in neonatal brain injury therapy. Based on data derived primarily from animal models, we analyze the heterogeneity across studies in model systems, administration routes, and efficacy endpoints, revealing common challenges in the field regarding long-term safety, manufacturability, and reproducibility. This review aims to provide guidance for selecting appropriate nanoplatforms to facilitate the translational advancement of this field toward clinical applications.
Keywords: neonatal brain injury, nanocarrier, neuroinflammation, oxidative stress

Introduction

Neonatal brain injury, primarily manifested as hypoxic-ischemic encephalopathy (HIE), neonatal stroke, and periventricular leukomalacia, represents a leading cause of infant mortality and long-term neurological sequelae (such as cerebral palsy, motor and cognitive impairments, and epilepsy).[1–3] HIE affects approximately 1 to 3 per 1000 live-born term infants, with an even higher incidence of brain injury among preterm neonates.[4] The pathophysiological cascade is initiated by perinatal hypoxic-ischemic (HI) insult, triggering a complex series of reactions characterized by a vicious cycle of excitotoxicity, oxidative stress, and neuroinflammation, ultimately leading to neuronal death, oligodendrocyte precursor cell damage, and myelination failure.[5,6]

Specifically, the pathophysiological cascade of neonatal brain injury involves multiple interconnected stages (Figure 1).[7] Initially, HI insult leads to the deprivation of energy substrates in brain tissue, shifting cellular metabolism from aerobic oxidation to anaerobic glycolysis, resulting in the rapid depletion of high-energy phosphate compounds such as ATP. This primary energy failure directly causes dysfunction of energy-dependent ion channels in cell membranes, leading to acute intracellular influx of sodium and calcium ions, cell membrane depolarization, and extracellular accumulation of the excitatory amino acid glutamate, which in turn triggers cytotoxic edema and acute necrotic cell death. This energy imbalance in the acute phase is the initiating factor that triggers the subsequent cascade of injury.[8–10] This calcium overload further impairs mitochondrial function, triggering the excessive generation of reactive oxygen species (ROS) and causing severe oxidative stress.[11] Concurrently, damaged cells release a multitude of damage-associated molecular patterns (DAMPs), which potently activate microglia and astrocytes, further drive a sustained neuroinflammatory response. Subsequently, activated microglia predominantly polarize towards a pro-inflammatory phenotype and release various pro-inflammatory cytokines and reactive nitrogen species (RNS). These

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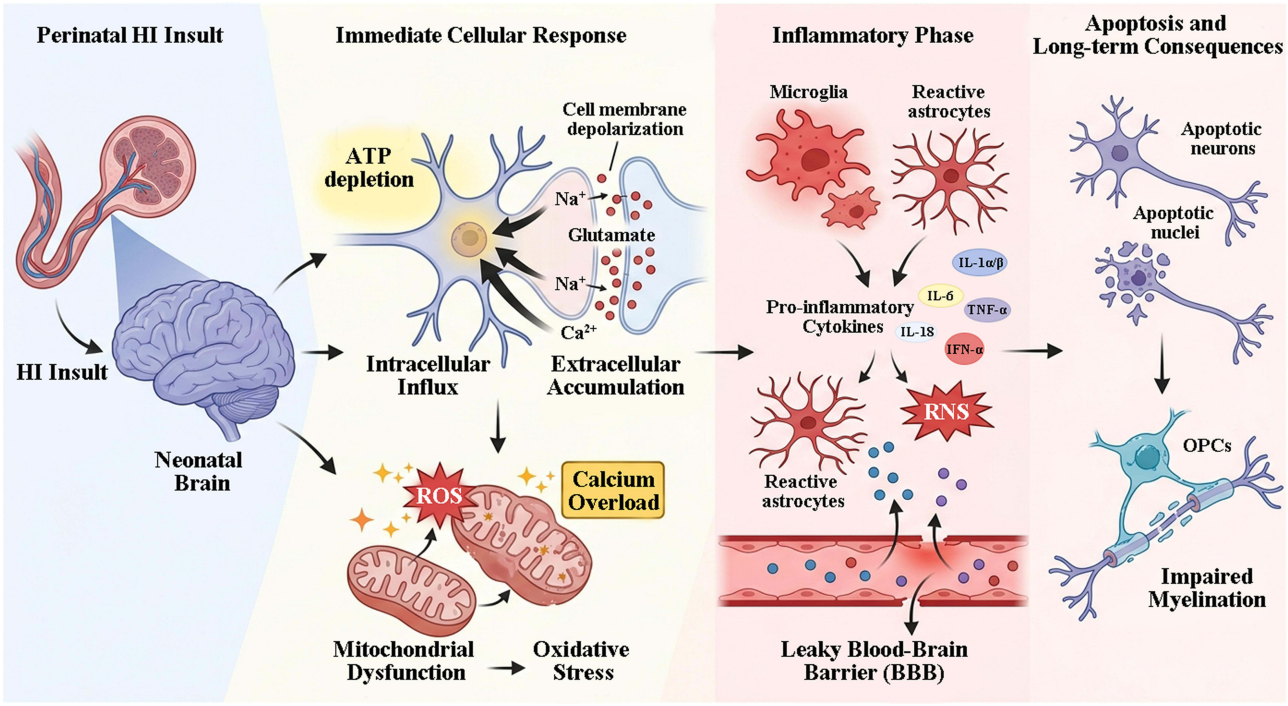

Figure 1 Schematic illustration of the pathophysiological cascade following perinatal hypoxic-ischemic (HI) insult. HI insult initiates primary energy failure, ATP depletion, and excitotoxicity, leading to calcium overload and mitochondrial dysfunction. This triggers excessive reactive oxygen species (ROS) production and oxidative stress. Concurrently, damaged cells activate microglia and astrocytes, driving neuroinflammation through pro-inflammatory cytokines and reactive nitrogen species (RNS) release. These factors compromise blood-brain barrier (BBB) integrity and propagate secondary injury characterized by apoptotic cell death, ultimately resulting in widespread neuronal loss and impaired myelination.
Abbreviation: OPCs, oligodendrocyte precursor cells.

factors combine with ROS, further exacerbating tissue damage and compromise the integrity of the blood-brain barrier (BBB). This cascade leads to the propagation of secondary injury, characterized by a second energy failure occurring hours to days after the initial insult, during which the majority of cell death occurs via apoptotic pathways, and ultimately resulting in widespread neuronal apoptosis and impaired myelination.[10,12–15]

Currently, therapeutic hypothermia stands as the only widely approved neuroprotective intervention for infants with HIE.[16] However, its application is constrained by a narrow therapeutic window (initiation within 6 hours after birth) and offers only partial neuroprotection,[17,18] with approximately 40% of treated neonates still experiencing death or significant neurodevelopmental impairment.[19,20] Recently, various emerging neuroprotective strategies are under clinical investigation, including anti-excitotoxic agents (eg, magnesium sulfate, topiramate), antioxidants (eg, allopurinol, melatonin), anti-inflammatory agents, and multi-target agents (eg, erythropoietin, stem cells).[19,21] However, most of these strategies face challenges such as poor BBB penetration, significant systemic side effects, or lack of neonatal-specific pharmacokinetic data.[22]

Nanomedicine presents a promising strategy for neonatal brain injury therapy. Nanomaterials usually refer to materials with a size ranging from 1 to 100 nm, and they leverage their unique size and engineerability to enable the targeted delivery of various therapeutic agents (including small molecule drugs, proteins, and nucleic acids), which significantly improve their pharmacokinetic profiles, enhance the accumulation in the injured brain regions, and reduce systemic side effects.[23–25] For instance, Kannan et al successfully delivered the anti-inflammatory drug N-acetylcysteine specifically to activated microglia using polyamidoamine (PAMAM) dendrimers, effectively attenuating white matter injury and neuroinflammation in a neonatal rabbit cerebral palsy (CP) model.[26] Another breakthrough study encapsulated mRNA within ionizable lipid nanoparticles (LNPs), which achieved highly efficient functional protein expression in the neonatal mouse brain following intracerebroventricular injection, opening a novel avenue for gene therapy of congenital brain disorders.[27]

Notably, the developmental maturation of the neonatal BBB is a dynamic process that critically influences nanocarrier design and brain targeting efficiency. Compared to adults, the neonatal BBB exhibits significant structural and functional differences. For example, the expression levels of tight junction proteins are not yet fully mature, resulting in relatively higher permeability to certain small molecules and macromolecules.[28,29] Concurrently, the expression of the efflux transporter P-glycoprotein (P-gp) is limited at birth, significantly influencing the brain distribution of substrate drugs.[30,31] Additionally, the extracellular matrix composition, water content, and cellular density of neonatal brain tissue differ from adults, collectively influencing nanoparticle diffusion and distribution within the brain parenchyma.[32] More critically, the low activity of hepatic metabolic enzymes and the low glomerular filtration rate in neonates substantially alter the systemic clearance kinetics of nanocarriers.[33] Therefore, the rational design of neonatal nanomedicines must integrate these developmental parameters rather than simply applying adult delivery strategies. Compared to other emerging neuroprotective approaches targeting excitotoxicity or apoptotic pathways,[34,35] nanocarriers possess the capacity to function as a multifunctional platform integrating targeted delivery, controlled release, and synergistic therapy. However, their clinical translation faces unique challenges, including neonatal-specific pharmacokinetic complexities and the need for rigorous long-term safety assessment.[36]

Current nanomaterial-based strategies for neonatal brain injury therapy primarily encompass four major categories (Figure 2). Polymer-based nanocarriers have been employed in neonatal brain injury therapy due to their prolonged systemic circulation and specific accumulation in injured brain areas.[37] Extracellular vesicles (EVs), as endogenous nanocarriers, have also been widely utilized to deliver bioactive cargo such as miRNAs, thereby modulating microglial polarization and significantly improving long-term neurodevelopmental outcomes in neonatal animal models.[38] Similarly, lipid-based nanocarriers can efficiently encapsulate and deliver mRNA to the perinatal brain, successfully achieving therapeutic base editing in neonatal animal models.[27] Furthermore, inorganic-based nanocarriers such as Prussian blue nanozymes possess intrinsic superoxide dismutase- and catalase-like activities, targeting neuronal mitochondria to directly scavenge excess ROS, effectively alleviating oxidative stress and promoting functional recovery in neuronal ischemic brain injury models.[39] Although the current efficacy data are derived exclusively from preclinical animal models, with no human clinical trial data currently available to support the clinical application of nanotherapies, these advances collectively underscore the potential of nanomedicine in neonatal brain injury therapy. This review aims to systematically elucidate the mechanisms, latest therapeutic approaches, and critically analyze the unique challenges associated with these four categories of nanocarriers in neonatal brain injury therapy.

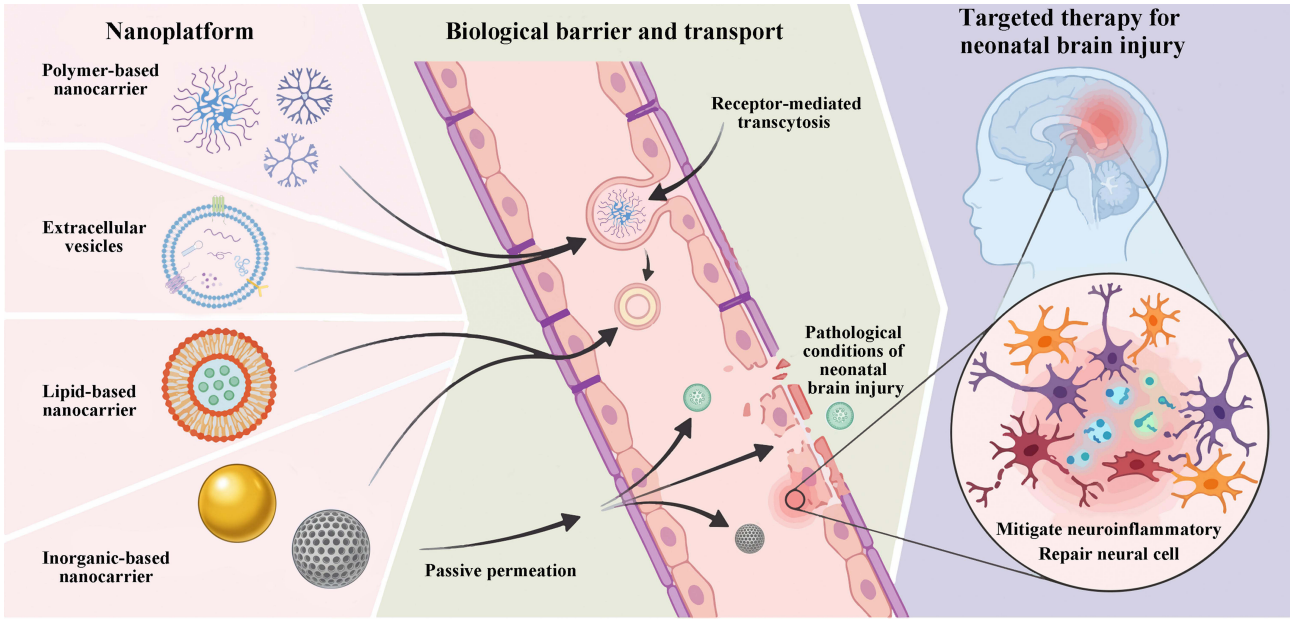

Figure 2 Schematic diagram of the main nanomedicine strategies for neonatal brain injury therapy.

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Polymer-Based Nanocarriers in Neonatal Brain Injury Therapy

Polymer-based nanocarriers constitute a class of nanoscale delivery systems constructed from natural or synthetic polymers through self-assembly or chemical synthesis. They exhibit highly tunable physicochemical properties and exceptional drug-loading versatility. By precisely designing the molecular weight, hydrophilicity/hydrophobicity, functional groups, and topology, researchers can achieve fine control over nanoparticle size, surface charge, degradation rate, and drug release kinetics can be achieved.[40,41] This tunability allows polymer-based nanocarriers to be tailored to the unique physiological environment of newborns and the complex pathological microenvironment following brain injury, such as local inflammation, acidosis, and oxidative stress.[42] Furthermore, polymer-based nanocarriers can efficiently load diverse therapeutic agents, including hydrophobic small molecules, hydrophilic macromolecular proteins, and even nucleic acids, thereby offering solutions to key challenges in neuroprotective drug delivery such as poor solubility, short half-life, and low brain distribution.[19]

The targeting and accumulation efficiency of polymer-based nanocarriers in the injured brain areas is determined by their physicochemical parameters, including the size, surface charge, and surface chemical modifications. It is suggested that nanoparticles with diameters in the 10–100 nm range are more favorable for penetrating the compromised yet still selective BBB.[43] And a near-neutral or slightly negative surface charge helps reduce non-specific adsorption to negatively charged plasma proteins, thereby prolonging systemic circulation and promoting passive accumulation in injured brain regions with increased vascular permeability via the enhanced permeability and retention (EPR) effect.[44] Additionally, polyethylene glycol (PEG) modification represents a classic method to extend the in vivo circulation time of polymer-based nanocarriers. For instance, PEGylated poly (lactic-*co*-glycolic) acid (PLGA) nanoparticles have demonstrated an extended half-life and higher brain accumulation in neonatal rats.[45] More importantly, active targeting strategies, achieved by chemically conjugating specific targeting ligands to the carrier surface, can significantly enhance targeting specificity.[46]

The therapeutic mechanisms of polymer-based nanocarriers in neonatal brain injury primarily revolve around their targeted delivery function. By precisely transporting therapeutic agents to specific cells at the injury core areas (eg, activated microglia, astrocytes, dying neurons, or oligodendrocyte precursor cells), they intervene in key pathological processes. Among these, modulating neuroinflammation and immune responses represents a principal mechanism. Polymer-based nanocarriers deliver anti-inflammatory molecules or biologics that inhibit inflammatory signaling pathways like the nuclear factor-kappa B (NF-κB) and p38 mitogen-activated protein kinase (MAPK) pathways,[47] thereby downregulating the expression of pro-inflammatory cytokines, and promoting the polarization of microglia from the pro-inflammatory M1 phenotype to the anti-inflammatory/reparative M2 phenotype, thereby breaking the vicious cycle of inflammation and creating a favorable environment for repair.[48,49] Secondly, these nanocarriers can alleviate oxidative stress and exert anti-apoptotic effects by delivering antioxidants or anti-apoptotic drugs. These agents directly neutralize excess ROS at the injury site, protect mitochondrial function, and inhibit apoptosis pathways such as the caspase cascade, thereby protecting neurons and oligodendrocytes from oxidative damage and programmed cell death.[50] Furthermore, some polymer-based nanocarriers, such as hydrogels, can serve as biomaterial scaffolds that fill injury cavities and provide physical support. Simultaneously, by sustainedly releasing neurotrophic factors or agents that recruit endogenous stem cells, they support neurite outgrowth, angiogenesis, oligodendrocyte maturation, and myelination, thereby initiating endogenous repair programs.[51]

Based on the engineerability of polymer-based nanocarriers, diverse therapeutic strategies have been developed to address the complex pathophysiology of neonatal brain injury. Long-acting delivery and targeted accumulation strategies form the foundation for achieving sustained neuroprotection. As a representative polymer platform, dendrimers possess highly branched three-dimensional structures and abundant surface functional groups, making them ideal drug carriers. The Kannan team first systematically investigated the biodistribution of generation-4 hydroxyl-functionalized PAMAM dendrimers (D4-OH) in a mouse model of ischemia-induced neonatal white matter injury, demonstrating their ability to passively target the injured region and undergo preferential uptake by activated astrocytes and microglia at different time points. A single administration of the dendrimer-N-acetyl cysteine conjugate (D-NAC) sustained the anti-inflammatory response for up to 9 days and improved myelination, pioneering the application of dendrimers for neonatal brain injury treatment.[52] In vitro mechanistic studies further confirmed that activated microglia exhibit significantly higher uptake of

D4-OH, correlating with enhanced inflammatory signaling in injured regions.[53] Building on this foundation, they employed generation-6 hydroxyl-terminated PAMAM dendrimers with extended circulation time (G6D-NAC) in a rabbit model of CP. A single intravenous injection not only achieved specific delivery to activated microglia, but also maintained motor function recovery until postnatal day 15,[37] highlighting the advantages of long-circulating carriers in achieving durable neuroprotection. However, the long-term in vivo safety of high-generation dendrimers remains a critical question requiring resolution prior to clinical translation. As another important polymer platform, PLGA-PEG nanoparticles exhibit formulation-dependent in vivo behavior. A study in full-term neonatal rats revealed that PLGA-PEG nanoparticles stabilized with Pluronic® F127 displayed a significantly longer half-life compared to those stabilized with Poloxamer 188, with the liver serving as the primary accumulation site and minimal brain uptake.[45]

For injuries requiring sustained local drug concentrations or involving tissue defects, implantable polymer scaffolds or hydrogels offer a unique solution. Lu et al developed a nanofibrous network constructed from self-assembling peptide $(RADA)_4$ and sulfobutyl ether β-cyclodextrin (SBE-β-CD) for loading dexamethasone. In a perinatal rat model of HI, this nanoscaffold enabled sustained release of dexamethasone, and local application effectively suppressed microglial activation and glial scar formation.[54] Similarly, Grebenik et al evaluated the compatibility of chitosan-*g*-oligo(L, L-lactide) copolymer hydrogel with primary cortical neurons, demonstrating its capacity to support cell survival and function under glutamate excitotoxicity, presenting a potential reparative scaffold for brain tissue engineering.[55] These strategies enable local, long-lasting drug release and may provide physical support. However, they typically require invasive implantation and offer limited coverage for diffuse injuries.

The versatility of polymer-based nanocarriers enables the delivery of diverse therapeutic agents and the exploration of novel administration routes. Xu et al applied curcumin-loaded polymeric nanoparticles to a fetal growth restriction (FGR) newborn piglet model, optimizing a nano-formulation with drug loading as high as 39%. They detected nanoparticles in the brain parenchyma, particularly in microglia, as early as 4 hours after intranasal administration, offering a non-invasive approach for treating FGR-associated neuroinflammation.[56] Joseph et al utilizing an ex vivo brain slice model, revealed how the pathological microenvironment influences nanocarrier behavior. They found that oxygen-glucose deprivation (OGD) injury significantly enhanced the diffusion of polystyrene-polyethylene glycol (PS-PEG) nanoparticles in tissue and their uptake by microglia, while azithromycin (AZ) treatment restored uptake to normal levels. This discovery provides a theoretical basis for designing intelligent nanocarriers responsive to pathological signals.[57] Shin et al employed PLGA nanoparticles to load the AMPA receptor antagonist perampanel. In a neonatal rat stroke model, intrathecal administration successfully induced microglial polarization toward the M2 phenotype, reduced pro-inflammatory factors, decreased infarct volume, and improved motor function, opening a new avenue for perinatal stroke treatment.[58] In the realm of protein drug delivery, researchers successfully encapsulated catalase within PLGA-PEG nanoparticles using hydrophobic ion pairing technology. This nano-formulation demonstrated significantly superior neuroprotection compared to controls in a neonatal rat model of HIE, offering a viable approach for enzyme therapy aimed at ROS scavenging.[59]

Extracellular Vesicles in Neonatal Brain Injury Therapy

EVs are nanoscale, lipid bilayer-enclosed vesicles actively secreted by cells,[60] serving as key mediators of intercellular communication, EVs carry and deliver bioactive molecules such as proteins, lipids, metabolites, and nucleic acids, thereby modulating gene expression and functional states.[61] This endogenous origin endows EVs with unique advantages as natural nanodrug delivery systems, including high biocompatibility, low immunogenicity, inherent ability to traverse biological barriers, and the potential to evade rapid clearance by the mononuclear phagocyte system.[62] In central nervous system diseases, EVs can not only serve as an acellular therapy exerting direct therapeutic effects but also function as intelligent carriers for the targeted delivery of therapeutic molecules.[63]

EVs derived from different cellular sources exhibit heterogeneous biological properties and therapeutic potentials. Currently, EVs used in neonatal brain injury are primarily sourced from mesenchymal stromal cells (MSCs), neural stem cells (NSCs), and brain tissue.[64–66] Among these, MSC-derived EVs are the most extensively studied and are enriched with miRNAs possessing immunomodulatory and neurotrophic properties and have been shown to confer protection in various brain injury models by regulating microglial polarization and suppressing neuroinflammation.[67] NSC-derived

EVs are thought to be more inclined towards promoting neurogenesis and neuronal survival.[68] Brain tissue-derived EVs may retain specific homing signals tailored to the central nervous system microenvironment.[66] Furthermore, to overcome the heterogeneity and expansion limitation of EVs from primary cells, EVs from immortalized or genetically engineered cells are being explored to achieve more standardized production and enhanced functionality.[38]

The core therapeutic mechanisms of EVs in neonatal brain injury stem from their role as natural information carriers, enabling them to intervene in crucial pathological processes through multiple pathways. Firstly, EVs modulate immune and inflammatory responses via the bioactive cargo (eg, miRNAs, proteins, and lipids) they carry. Within the injured brain, EVs can be taken up by microglia, astrocytes, and neurons, further delivering immunomodulatory miRNAs. These molecules, by inhibiting signaling pathways such as STAT3 and JMJD3/p53, promote the transition of pro-inflammatory M1-type microglia toward an anti-inflammatory M2 phenotype, thereby effectively mitigating neuroinflammation.[69–71] Secondly, EVs possess antioxidant and anti-apoptotic properties. miRNAs or enzymes carried by EVs can enhance cellular antioxidant capacity, inhibit excessive ROS production, and downregulate the expression of apoptosis-related proteins like caspase-3, thereby protecting neurons and oligodendrocytes from oxidative damage and programmed cell death.[70] Recent studies have further revealed that EVs from specific sources, such as small EVs (sEVs) derived from hypoxia-preconditioned MSCs, exert neuroprotective effects by regulating the SIRT1/Nrf2/HO-1 signaling pathway and enhancing the activity of endogenous antioxidant enzymes including superoxide dismutase (SOD).[72] Additionally, EVs can promote neural repair and regeneration by enhancing neurogenesis, angiogenesis, oligodendrocyte maturation, and myelination by delivering pro-growth factors or activating endogenous NSCs, thereby supporting post-injury brain tissue repair.[38,73] Collectively, these mechanisms form the basis for the multi-target intervention of EVs in the brain injury cascade.

Current application of EVs in neonatal brain injury therapy primarily manifests in two approaches. Among these, the strategy of utilizing the endogenous bioactive components of EVs, particularly miRNAs have been the most extensively studied, with detailed mechanistic elucidation. For instance, a study using an ovine fetus hypoxia-ischemia model first demonstrated that systemic administration of MSC-EVs significantly improved cerebral electrophysiological function and showed a trend toward protecting myelination, highlighting their direct neuroprotective potential in a large neonatal animal model.[67] Subsequent research has focused on miRNAs carried by MSC-EVs, for example, Xin et al found that MSC-EVs deliver miR-21a-5p to microglia in the injured brain region. miR-21a-5p drives microglial polarization toward an anti-inflammatory M2 phenotype by inhibiting the STAT3 signaling pathway, thereby alleviating neuroinflammation and acute brain damage in neonatal mouse models.[69,74] Similarly, Luo et al confirmed that bone marrow MSC-EVs deliver miR-93, which targets and inhibits JMJD3, thereby regulating the downstream p53/KLF2 axis, ultimately exerting anti-apoptotic and neuroprotective effects in both hippocampal neurons and a neonatal mouse model of HI brain injury.[70]

Beyond MSCs, EVs from other cellular sources also demonstrate therapeutic potential due to their unique active components. For example, Nguyen et al innovatively isolated EVs directly from whole brain rat tissue (BEV). They demonstrated in an ex vivo OGD brain slice model that BEVs could reduce cytotoxicity and simultaneously promote microglial shift toward an anti-inflammatory phenotype, suggesting that brain tissue may release EVs with endogenous protective signals after injury.[66] Another study compared NSC-EVs with EVs from hypoxia-preconditioned brain cells (brain-EVs) and found that intranasal administration of either could reduce cerebral infarct volume in neonatal mice. However, NSC-EVs more significantly reduced total apoptotic cells, whereas Brain-EVs were more effective in down-regulating caspase-3 expression.[65] These studies indicate that EVs from different sources may function through distinct mechanistic pathways, offering the possibility of selecting optimal EV sources for specific pathological stages. Nonetheless, systematic knowledge regarding the biological characteristics, long-term safety, and scalable production of these non-mainstream EV sources remains unclear.

To enhance the efficacy and overcome heterogeneity, recent studies have focused on engineering EVs and optimizing therapeutic strategies. Labusek et al combined EVs from clonally expanded immortalized MSCs (ciMSC-EVs) with the therapeutic hypothermia. In a neonatal mouse hypoxia-ischemia model, intranasal delivery of ciMSC-EVs not only compensated for the limitations of hypothermia alone, significantly reducing acute-phase neuroinflammation and neuronal loss, but more crucially, promoted endogenous regenerative processes (such as endothelial cell proliferation

and neurotrophic factor expression) that were not effectively stimulated by hypothermia alone, thereby synergistically improving long-term neurobehavioral outcomes.[38] This study partially addresses the standardization challenge in EV production through the use of an immortalized cell source, however, the potential tumorigenic risk associated with immortalized cells requires stringent evaluation. Furthermore, Kaminski et al systematically confirmed the multifaceted benefits of MSC-EVs by administering them via intraperitoneal injection at multiple time points after hypoxia-ischemia in neonatal mice. They demonstrated that MSC-EVs could not only modulate the activation states of microglia and astrocytes, shifting the cytokine profile toward an anti-inflammatory direction, but also significantly promote neural cell proliferation and angiogenesis within the brain.[73] This study enriches the evidence for MSC-EVs in promoting endogenous repair from a multi-timepoint, multi-target perspective. However, the intraperitoneal injection route may not be the optimal choice for neonatal clinical applications in terms of convenience and acceptability.

Lipid-Based Nanocarriers in Neonatal Brain Injury Therapy

Lipid-based nanocarriers, primarily comprising liposomes and LNPs, constitute a class of nanoscale delivery systems constructed mainly from phospholipids, cholesterol, and synthetic lipids. Their core lipid bilayer structure endows them with high biocompatibility and biodegradability akin to biological membranes, forming the cornerstone of their utility as drug carriers.[75] In neonatal brain injury therapy, the exceptional drug encapsulation versatility and engineerability surface properties of lipid-based nanocarriers enable them to efficiently load a diverse array of therapeutic agents, ranging from hydrophobic small molecules and hydrophilic drugs to macromolecular nucleic acids such as mRNA and miRNA.[76] For instance, liposomes have been widely used to deliver small-molecule drugs and proteins, while LNPs based on ionizable lipids have revolutionized the in vivo delivery of nucleic acid therapeutics.[77] Furthermore, their surface characteristics and in vivo fate can be precisely modulated by incorporating PEG-lipids to prolong circulation time or by conjugating specific targeting ligands such as peptides or antibodies.[78,79] This engineering capability is crucial for navigating the dynamic changes in the developing BBB of neonates and for achieving active targeting to specific sites such as activated glial cells in injured brain regions.[19]

The therapeutic mechanism of lipid-based nanocarriers in neonatal brain injury is mediated primarily through the active substances they deliver, which intervene in several core pathological processes.[80] Firstly, by delivering neuroprotective hormones or anti-inflammatory drugs, the overactivation of microglia and astrocytes is inhibited, thereby reducing the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), which mitigates secondary inflammatory damage.[81] Secondly, the delivery of mitochondria-targeted antioxidant molecules or gene-regulating nucleic acids can directly alleviate oxidative stress and regulate apoptotic pathways. This involves scavenging excess ROS and preserving mitochondrial membrane potential, thereby protecting vulnerable neurons and oligodendrocyte precursor cells from oxidative damage and programmed cell death.[82,83] Additionally, certain bioactive factors, such as vascular endothelial growth factor (VEGF), can promote angiogenesis and endogenous repair, providing essential nutritional and oxygen support for post-injury neural tissue recovery and potentially activating endogenous neurogenesis.[84]

A primary therapeutic strategy in neonatal brain injury involves using lipid-based nanocarriers to improve the pharmacokinetics of neuroprotective small molecules. For example, to address the delivery challenges of estetrol (E4), a study compared different formulations and found that in a neonatal rat model of HIE, E4 encapsulated within a drug-in cyclodextrin in liposome (DCL) system (DCL-E4) was more effective than free E4 or conventional liposomes in preserving brain tissue, reducing glial fibrillary acidic protein (GFAP) release, and specifically promoting hippocampal angiogenesis.[85] In another study focusing on the targeted delivery of the hydrogen sulfide (H_2S), the mitochondria-targeted H_2S donor AP39 was encapsulated into liposomes (AP39@Lip) and administered via the intranasal route. In a neonatal mouse model of HI, AP39@Lip specifically localized to neuronal mitochondria. By inhibiting the ERK1/2 and caspase-1 pathways, it effectively mitigated mitochondrial dysfunction, apoptosis, and neuroinflammation, while improving long-term neurological function.[86] These studies demonstrate the capacity of lipid-based nanocarriers to enhance drug delivery efficiency and achieve targeted therapy, though the scalability of their large-scale production requires further validation.

Recently, therapeutic nucleic acids have also been incorporated into lipid-based nanocarriers for developing strategies to treat neonatal brain injury. For instance, Song et al employed intranasal delivery of liposome-encapsulated miRNA mimics. They found that miR-34c-5p expression was downregulated after HI injury, and that treatment with its liposomal mimic significantly alleviated brain damage, oxidative stress, and inflammation, thereby promoting functional recovery by targeting GTPase activating protein 26 (*Arhgap26*).[87] Similarly, Zhang et al demonstrated that a liposome-delivered miR-128-3p mimic exerted comparable neuroprotective effects in HI neonatal mice by inhibiting Regulating G protein signaling 1 (*Rgs1*).[88] While intranasal liposomal delivery overcomes the challenges of nucleic acid degradation and low brain delivery efficiency, the multi-target nature and long-term safety of miRNA therapeutics necessitate further in-depth investigation. Furthermore, a breakthrough in mRNA therapy and gene editing was achieved by Palanki et al They optimized a novel LNP to deliver mRNA encoding an adenine base editor to the neonatal mouse brain. This platform achieved high-efficiency protein expression and gene editing in the brain and demonstrated its potential in a fetal non-human primate model via intracerebroventricular injection.[27]

Beyond the development of strategies for delivering therapeutic agents, fundamental formulation science and systematic safety assessment are also critical. Palazzo et al systematically developed injectable E4 liposome and DCL formulations intended for the prevention of cerebral ischemia in premature infants. They comprehensively evaluated the physicochemical properties, stability, biocompatibility, and ability of these formulations to penetrate an in vitro BBB model, thereby laying a solid foundation for subsequent in vivo studies.[89]

Inorganic-Based Nanocarriers in Neonatal Brain Injury Therapy

In neonatal brain injury therapy, inorganic-based nanocarriers are evolving beyond their traditional role as passive drug carriers to function as therapeutic agents with intrinsic biological activity. Composed of metals, metal oxides, or metal salts, these nanomaterials offer tunable size and morphology, high stability, and, most importantly, enzyme-mimicking (nanozyme) ability.[90,91] This intrinsic property enables them to directly catalyze the clearance of excess ROS produced during injury, thereby intercepting the vicious cycle of oxidative stress, representing a sustained and efficient catalytic capability that many small-molecule antioxidants lack.[92] Furthermore, several inorganic-based nanocarriers possess excellent magnetic or optical properties, enabling applications like magnetic resonance imaging (MRI) or optical imaging, thus offering potential for combined therapy and diagnosis applications.[93] Currently, inorganic-based nanocarriers applied in neonatal brain injury research mainly include noble metal nanoclusters, transition metal oxide nanoparticles, and Prussian blue analogues, which exert neuroprotective effects through distinct catalytic mechanisms.

The core therapeutic mechanisms of inorganic nanocarriers in neonatal brain injury primarily stem from their potent antioxidant and anti-inflammatory effects. As nanozymes, they can mimic the activity of endogenous antioxidant enzymes such as SOD, catalase (CAT), and glutathione peroxidase (GPx), converting harmful ROS into harmless water and oxygen. This directly protects neurons and oligodendrocytes from oxidative damage.[94,95] Research has demonstrated that nanozymes based on metal-phenolic networks, such as curcumin-copper complex nanoparticles (Cur-Cu NPs), exhibit SOD-mimetic activity, effectively scavenging excess ROS and providing cytoprotective effects by inhibiting the caspase-3-dependent apoptotic pathway.[96] Secondly, by alleviating oxidative stress and directly interacting with inflammatory signaling molecules, they can inhibit the overactivation of microglia, downregulate the expression of pro-inflammatory cytokines, and thereby indirectly mitigate neuroinflammation.[97] More importantly, advanced material design enables the precise delivery of these nanozymes to subcellular organelles, such as mitochondria, allowing for intervention at the very source of oxidative damage, significantly enhancing therapeutic efficiency while reducing off-target effects.[98] Some inorganic-based nanocarriers also exhibit supplementary functions like anti-apoptosis and promotion of angiogenesis, collectively supporting tissue repair.[99]

Therapeutic strategies based on inorganic nanocarriers mainly focus on enhancing their catalytic efficiency, improving brain targeting, and exploring theranostic capabilities. For instance, in exploring the application of glutathione-protected Au_{22} nanoclusters (GSH-Au_{22} NCs) in neonatal brain injury, Zheng et al found that in a neonatal rat HI model, GSH-Au_{22} NCs significantly reduced cerebral infarct volume, attenuated inflammation and oxidative stress, and improved long-term learning and memory. Further mechanistic studies revealed that their protective effect was achieved by upregulating the Sirt3/SOD2 signaling pathway. Sirt3, a mitochondrial deacetylase, activates the antioxidant enzyme

SOD2, thereby enhancing the cell's intrinsic antioxidant defenses.[95] This study is the first to reveal that ultrasmall gold nanoclusters exert neuroprotection by modulating specific mitochondrial signaling pathways, providing new evidence for noble metal nanomaterials playing a role beyond mere drug delivery in neuroscience.

Regarding the theranostic application of inorganic nanocarriers in neonatal brain injury, Jeon et al employed hollow manganese oxide nanoparticles (HMONs) as T_1-weighted MRI contrast agents for the non-invasive, dynamic monitoring of apoptosis following hypoxic-ischemic brain injury in neonatal rats. HMONs produced specific enhancement signals in brain regions with active apoptosis (eg, dorsolateral thalamus, hippocampus) for up to 21 days post-injury, and this enhancement showed strong spatial correlation with apoptotic cells identified by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining.[100] This work provides a crucial tool for assessing therapeutic efficacy. However, its definitive independent therapeutic efficacy requires further validation, and the long-term neurological effects of manganese ions need careful evaluation.

To overcome the critical challenge of insufficient accumulation of nanozymes in diseased brain regions, researchers have developed surface engineering strategies to enhance their targeted delivery efficiency. Among these, polydopamine (PDA) coating has garnered significant attention due to its excellent biological adhesive and reactive properties. Studies have demonstrated that coating Prussian blue nanoparticles with SOD and CAT activities with a PDA shell (PB@PDA NPs), not only significantly improves the colloidal stability and biocompatibility of the nanoparticles, but also endows them with the ability to target neuronal mitochondria. In a neonatal mouse HI model, compared to unmodified PB NPs, PB@PDA NPs exhibited significantly higher brain accumulation and more effectively localized to neuronal mitochondria, thereby achieving more efficient ROS scavenging, inhibition of apoptosis and inflammation, and promotion of both short-term and long-term functional recovery.[39] This strategy simultaneously addresses the challenges of stability, brain delivery, and organelle targeting, offering a valuable reference for the intracerebral application of nanozymes.

In parallel with surface modification strategies, another important direction involves optimizing administration routes to achieve non-invasive delivery. Jiang et al developed a cerium vanadate ($CeVO_4$) nanozyme with SOD activity and innovatively employed the intranasal administration route, successfully bypassing the limitations of the blood-brain barrier. Their study confirmed that intranasally administered $CeVO_4$ nanozyme effectively reached the brain parenchyma and preferentially localized to neuronal mitochondria. In a neonatal mouse HI model, this therapeutic strategy significantly alleviated acute-phase injury and promoted long-term neurological functional recovery, with its protective effects closely associated with activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant pathway.[101] By integrating a highly efficient nanozyme with a non-invasive delivery method, this study greatly enhanced treatment accessibility and potential patient compliance.

Challenges in Clinical Translation

Despite the promising therapeutic potential demonstrated by nanomedicine in preclinical studies of neonatal brain injury, advancing these strategies toward clinical translation necessitates a prudent evaluation of their limitations in toxicity, manufacturability, regulatory hurdles, and reproducibility. As summarized in Table 1, significant heterogeneity exists across different nanotherapeutic strategies in terms of model systems, administration route, and efficacy endpoints. This heterogeneity directly impacts the robustness and comparability of research conclusions and reveals the core obstacles hindering the evolution of nanomedicine toward a unifying therapeutic paradigm.

Long-term safety and toxicity considerations represent the foremost challenge in the clinical translation of nanomedicine, particularly for the developing neonatal brain. Currently, safety assessments in most studies are confined to acute-phase observations, with systematic investigations into the long-term effects of nanomaterials during critical developmental windows largely absent. For instance, while high-generation PAMAM dendrimers enable prolonged circulation and targeted delivery, their biodegradability and metabolic fate in vivo remain to be elucidated, raising concerns that chronic accumulation may trigger unknown immunogenicity or neurotoxicity.[37] Similarly, although inorganic nanomaterials such as HMONs have enabled dynamic apoptosis monitoring for up to 21 days, the accumulative effects and potential neurotoxicity of their degradation product Mn^{2+} in the developing brain have not been adequately evaluated.[100] Regarding lipid-based nanocarriers, while the success of LNPs in mRNA vaccines provides some safety precedent, their

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Table 1 A Comprehensive Comparison of the Main Nanocarriers Applied in Preclinical Studies of Neonatal Brain Injury

Nanocarrier	Model System	Administration Stage	Administration Route	Main Efficacy Endpoints	Advantages	Limitations	Reference
Polymer-based nanocarriers							
D-NAC	Mouse model of ischemia-induced neonatal white matter injury	Neonatal (six-day old (P6) or P10)	Intraperitoneal injection (i.p.)	Attenuated the pro-inflammatory response, improving myelination.	1. Engineerability: The molecular weight, topological structure and surface functional groups can be precisely regulated; 2. Flexible drug loading, capable of carrying small molecule drugs (NAC) as well as large molecular proteins; 3. Controlled release: sustained or stimuli-responsive release; 4. Active targeting: facile surface ligand modification.	1. Unknown long-term biosafety of high-generation dendrimers; 2. Accumulation risk of non-biodegradable polymers; 3. Batch-to-batch consistency challenges in scale-up; 4. Lack of neonatal-specific pharmacokinetic data; 5. Individual variability in BBB penetration efficiency.	[52,53]
G6D-NAC	Rabbit model of cerebral palsy (CP)	Neonatal (postnatal day 1 (PND1))	Intravenous injection (i.v.)	Significantly reduced neuroinflammation and rescued motor function to nearly healthy control levels at PND15.			[37]
$(RADA)_4$/SBE-β-CD nanoscaffold	Rat perinatal HI model	Perinatal	Intracerebral implantation	Sustained dexamethasone release, inhibited microglial activation and glial scar formation.			[54]
Chitosan-g-oligo(L,L-lactide) copolymer hydrogel	Primary cortical neuron culture	Neonatal	In vitro co-culture	Supported cell survival and function under glutamate excitotoxicity.			[55]
Curcumin-loaded polymeric nanoparticles	FGR piglet model	Neonatal (P1)	Intranasal (i.n.) and i.v.	Nanoparticles detected in brain parenchyma at 4h, and colocalized to microglia			[56]
PLGA encapsulating perampanel	Neonatal rat stroke model	Neonatal (P7)	Injected intrathecally	Induced microglial M2 polarization, reduced pro-inflammatory factors, decreased infarct volume, improved motor function.			[58]
PLGA-PEG encapsulating catalase	Neonatal rat HIE model	Neonatal (P10)	i.v.	Significant neuroprotective effect.			[59]
Extracellular vesicles (EVs)							
MSC-derived EVs	Fetal sheep HI model	Fetal	i.v.	Improved cerebral electrophysiological function, reduced seizure burden, trend toward myelination protection	1. Natural biocompatibility: EVs are of endogenous origin and have low immunogenicity; 2. Intrinsic bioactivity: carry active substances such as miRNAs, proteins that directly modulate cellular functions; 3. Having a natural tropism to injured tissues and the ability to cross the blood-brain barrier.	1. The isolation, purification, and characterization methods are not unified, posing challenges for large-scale production; 2. The composition of EVs cargo from different sources and batches varies greatly; 3. Lack of standardized dosage units, and long-term mechanism in vivo is still unclear.	[67]
MSC-derived EVs (miR-21a-5p)	Neonatal mouse HI model	Neonatal (P8)	Intracardial injection	Induced microglial M2 polarization by inhibited STAT3 pathway, attenuated neuroinflammation and acute brain injury.			[69,74]
BMSC-derived EVs (miR-93)	Hippocampal neuron models of OGD, neonatal mouse HIBD model	Neonatal (P7)	Co-culture, intranasal administration	Inhibited the hippocampal neurons apoptosis induced by OGD, reduced brain injury by regulating JMJD3/p53/KLF2 axis.			[38]
Brain tissue-derived EVs (BEVs)	Ex vivo brain slice OGD model	Neonatal	In vitro incubation	Reduced cytotoxicity and promoted microglial shift to anti-inflammatory phenotype.			[73]
NSC-derived EVs (NSC-EVs), hypoxia-preconditioned brain cell-derived EVs (brain-EVs)	Neonatal mouse HIBI model	Neonatal (PND9)	Intranasal administration	Reduced infarct size, NSC-EVs significantly decreased TUNEL+ cells, brain-EVs downregulated caspase-3 expression.			[70]
Immortalized MSC-derived EVs (ciMSC-EVs)	Neonatal mouse HI model	Neonatal (P10, P12, P14)	Intranasal administration	Reduced neuroinflammation, promoted endothelial proliferation and neurotrophic factor expression, and improved long-term cognitive behavior.			[65]
MSC-derived EVs	Neonatal mouse HI model	Neonatal (P9)	Intraperitoneal injection	Modulated glial activation, shifted cytokine profile toward anti-inflammatory, and promoted neural cell proliferation and angiogenesis.			

Lipid-based nanocarriers							
DCL-E4	Neonatal rat HIE model	Neonatal (P7)	i.p.	Preserved gray and white matter loses, upregulated hippocampal angiogenesis.	1. Composed of phospholipids, with excellent biocompatibility; 2. Flexible drug loading: encapsulating hydrophilic/hydrophobic drugs, suitable for nucleic acid delivery, and achieve intracellular delivery; 3. Facile surface modification: PEGylation for prolonged circulation, ligand conjugation for targeting.	1. Liposomes prone to oxidation, storage stability limited, unmodified liposomes easily phagocytosed by RES 2. Low encapsulation, especially for hydrophilic drugs; 3. The potential neonatal-specific toxicity, cationic lipids require safety assessment, and the safety of lipid in the developing brain is unknown.	[85]
AP39@Lip	Neonatal mouse HI model	Neonatal (P7-9)	Intranasal administration	Inhibited ERK1/2 and caspase-1, attenuated mitochondrial dysfunction, apoptosis, and inflammation.			[86]
miR-34c5p mimics@Lip	Neonatal mouse HI model	Neonatal (P6)	Intranasal administration	Attenuated apoptosis, oxidative stress, and inflammation, improved short-term and long-term neurological function.			[87]
miR-128-3p@Lipo	Neonatal mouse HI model	Neonatal (P7)	Intranasal administration	Ameliorated cerebral infarcts, apoptosis, neuroinflammation, and ROS levels, and facilitated the recovery of neurological function.			[88]
Inorganic-based nanocarriers]							
GSH-Au_{22} NCs	Neonatal rat HI model, primary neuron OGD model	Neonatal (P7)	i.p.	Reduced cerebral infarction volume, attenuated inflammation and oxidative stress, improved learning and memory, upregulated Sirt3/SOD2 pathway.	1. Intracellular catalytic activity: For instance, as a catalytic therapeutic agent, nanoenzymes directly eliminate ROS; 2. Their ultra-small size facilitates renal clearance and reduces accumulation; 3. Possessing unique mechanisms, such as the ability to regulate endogenous antioxidant signaling pathways.	1. Although inorganic nanomaterials can be cleared by the kidneys, the tissue distribution after long-term exposure is unknown; 2. The atomic-level synthesis is difficult and the yield is low; 3. The degradation and metabolism pathways in the developing brain are not clear.	[95]
HMON	Neonatal rat HII model	Neonatal	i.p.	MRI dynamic monitoring of apoptotic regions for up to 21 days, and consistent with TUNEL staining.			[100]
PB@PDA NPs	Neonatal mouse HI model	Neonatal	Intracardial injection	Attenuated oxidative stress, inhibited apoptosis and inflammation, promoted short-term and long-term functional recovery			[39]
$CeVO_4$	Neonatal mouse HI model	Neonatal (P7-9)	Intranasal administration	Attenuated acute phase injury, promoted long-term functional recovery, and activated Nrf2 pathway.			[101]

potential pro-inflammatory effects in the neonatal immune system, as well as the direct toxicity of cationic lipids to developing neurons require dedicated evaluation.[27,87]

Even with EVs, despite their inherent biocompatibility, caution is warranted regarding their bidirectional regulatory effects, which may exacerbate neuroinflammation rather than promote repair under certain conditions.[102] The functional effects of EVs are highly dependent on the activation state of their parent cells, the specific cargo molecules they carry, and the pathological microenvironment.[103,104] For instance, activated microglia can release pro-inflammatory EVs via the CCR5-GPCRs-Ras-MAPK pathway, thereby aggravating neuroinflammation.[105] EVs carrying serum amyloid A1 have been shown to worsen neurological damage following intracerebral hemorrhage.[102] Furthermore, EVs released upon LPS stimulation can propagate neuroinflammation to healthy tissue through the opening of Cx43 hemichannels and the induction of aberrant calcium signaling in astrocytes.[106] However, it is noteworthy that all EVs discussed in this review for the treatment of neonatal brain injury demonstrated protective effects by attenuating neuroinflammation. This discrepancy likely arises because the EVs employed in these studies were primarily derived from MSCs rather than microglia in a pro-inflammatory state. The anti-inflammatory molecules such as miR-21a-5p and miR-93 inherently carried by these EVs can actively induce microglia polarization towards the anti-inflammatory M2 type by inhibiting signaling pathways such as STAT3 and JMJD3/p53.[38,74] Additionally, the specific EV dosages, isolation methods, and routes of administration used in these studies likely favored their protective functions over potential detrimental effects. This phenomenon highlights that the therapeutic efficacy of EVs is not an intrinsic, unchangeable property, but is instead highly dependent on their cellular source, preparation methods, and administration protocols. Notably, a clinical trial involving exosomes in the newborns (NCT05490173) was initiated in 2022, which aim to evaluate the safety and long-term neurodevelopmental outcomes of intranasally administered MSC-derived EVs in extremely low birth weight infants,[47] marking a critical step in the translational journey of this field from preclinical research to clinical application.

Manufacturability and standardization challenges constitute the second barrier to clinical translation. Substantial disparities exist across studies in nanocarrier preparation techniques, characterization methods, and quality control measures. In the case of EVs, studies employ diverse isolation methods and adhere to varying characterization standards, directly leading to batch-to-batch variations in EV cargo composition and bioactivity, thereby hindering reliable replication of therapeutic efficacy.[38,69,70] Although polymer-based nanocarriers offer the advantage of high engineer-ability, batch-to-batch consistency in their complex multi-step synthesis processes poses a bottleneck for large-scale production. As demonstrated, surfactant selection alone can significantly influence the half-life of PLGA nanoparticles in neonatal rats.[45] Liposome and LNP preparation face analogous challenges, for example, while the DCL system enhances drug loading, its complex multi-compartment structure may introduce new stability variables.[85]

Furthermore, pharmacokinetic and toxicological data for the vast majority of nanomedicines are derived from adult animal models or adult clinical studies. However, the distinct physiological environment of newborns including dynamic changes in blood-brain barrier development, immature hepatic and renal function, and an incompletely developed immune system, precludes simple extrapolation of adult experience to the pediatric population. As shown in Table 1, while most studies validate efficacy in neonatal animal models, the diversity in species (mouse, rat, rabbit, sheep, piglet), postnatal ages (ranging from P1 to P10), and injury models (HI, CP, stroke, FGR) complicates cross-study comparisons and poses challenges for regulatory agencies in establishing unified preclinical evaluation standards. For instance, although the fetal sheep model offers greater clinical relevance, its high cost and operational complexity limit widespread application in early-stage screening.[67] Moreover, the potential off-target effects of nucleic acid therapeutics during critical neurodevelopmental windows remain a concern, and there is currently a lack of clear regulatory guidelines mandating systematic assessment.[87,88]

Finally, even within the same platform, variations in administration routes (intravenous, intraperitoneal, intranasal, intrathecal, intracerebral implantation) and efficacy endpoints (acute histological vs. long-term behavioral outcomes) render study results difficult to directly compare and synthesize. For example, in EVs research, although both Labusek et al and Kaminski et al demonstrate neuroprotective effects of MSC-EVs, differences in cell sources, administration routes, and efficacy endpoints preclude quantification of effect sizes and establishment of optimal therapeutic protocols.[38,73] This heterogeneity stems not only from variations in experimental design but also reflects a potential tendency toward selective reporting of positive findings. Most studies lack multi-center validation, making reports from single research teams susceptible

to operator bias. Most critically, the absence of long-term safety follow-up hinders accurate assessment of therapeutic windows and risk-benefit ratios. The vast majority of studies observe outcomes for no more than one month postnatally, whereas long-term effects on neurodevelopment may extend into adolescence and even adulthood.[39,101]

In summary, while the nanocarriers mentioned above demonstrate multi-faceted, multi-target intervention potential in neonatal brain injury therapy, current research remains in the transitional phase from proof-of-concept to preclinical optimization. Advancing toward a unifying therapeutic paradigm necessitates confronting the systemic challenges in toxicity, manufacturability, regulatory frameworks, and reproducibility. This requires the field to establish standardized protocols, promote multi-center validation, and prioritize long-term safety studies encompassing developmental toxicity.

Conclusion and Outlook

The treatment of neonatal brain injury has long faced significant challenges due to the complexity of its pathological mechanisms, the narrow therapeutic window, and the limitations imposed by the BBB.[107] Nanomedicine presents a revolutionary opportunity for this field, with its unique ability to effectively traverse or exploit the compromised BBB to deliver therapeutic agents to injured brain regions inaccessible to conventional drugs.[108] Among various nanoplatforms, polymer-based nanocarriers like PAMAM dendrimers and PLGA nanoparticles enable the effective delivery of both small-molecule drugs and macromolecular proteins through precise targeting design and controlled drug release, significantly extending the therapeutic window and enhancing efficacy.[19,37,59] EVs, particularly exosomes derived from MSCs exhibit unique advantages in modulating microglial polarization and mitigating neuroinflammation, capitalizing on their innate biocompatibility, low immunogenicity, and inherent targeting capabilities.[38] Lipid-based nanocarriers, especially LNPs have successfully overcome the bottleneck of intracerebral nucleic acid drug delivery, opening a novel avenue for gene regulation and gene editing therapies for congenital brain disorders.[27] Meanwhile, inorganic-based nanocarriers particularly multi-enzyme mimetic nanozymes function as catalytic therapeutics, providing a potent tool against the core pathological process of oxidative stress by directly scavenging ROS and targeting mitochondria.[109]

However, the clinical translation of nanomedicine in the neonatal population faces a series of formidable challenges. First, the developing neonatal brain exhibits unique vulnerability. Compared to adults, neonates have higher BBB permeability, incomplete myelination, and active neural precursor cells, which amplify the potential neurotoxic risks of nanomaterials. The long-term biocompatibility, potential immunogenicity, toxicity of degradation products, and accumulation effects of nanomaterials in non-target organs (eg, liver, spleen) are completely different from those in adults against the background of immature neonatal hepatic and renal function in newborns,[36,45] necessitating systematic and rigorous evaluation according to pediatric standards. Second, challenges exist in standardization and scalable manufacturing. Whether the standardization of isolation, purification, and characterization for EVs, or the complex production processes for gene therapy LNPs, there remains a significant gap in meeting stringent Good Manufacturing Practice (GMP) requirements.[38,89] More critically, neonatal formulations require extremely precise dosing, for which current production systems lack targeted design. Furthermore, there are limitations regarding the clinical relevance of disease models. Although neonatal rodent models are widely used, differences in brain developmental stage, injury mechanisms, and immune systems compared to human preterm or term infants may affect the accuracy of efficacy predictions.[67] Large animal models like piglets and sheep, while more clinically relevant, are costly and lack standardization, limiting their widespread application.[110] Finally, there is a paucity of pharmacokinetic studies specific to the neonatal population. Neonates have markedly different hepatic and renal functions, plasma protein composition, and BBB status compared to adults. Currently, pharmacokinetic data for most nanomedicines are derived from adult animal models, lacking systematic research tailored to neonatal physiology.[85]

In the future, the development of nanomedicine strategies for neonatal brain injury should focus on the following directions:

(1) Advancing combination therapy strategies: Exploring the integration of nanocarriers with existing standard therapies such as therapeutic hypothermia or co-delivering different drugs with complementary mechanisms of action (eg, anti-inflammatory agents combined with neurotrophic factors), is expected to rapidly advance to preclinical optimization stages.

(2) Optimizing non-invasive or minimally invasive administration routes: Non-invasive routes, such as intranasal delivery, can improve patient compliance and potentially enable direct brain delivery, offering significant advantages for clinical translation. Research on their delivery efficiency, mechanisms, and safety should be intensified, along with the development of specialized nano-formulations suitable for these routes.
(3) Leveraging Clinically Validated Platforms: LNPs, benefiting from the success of mRNA vaccines, possess mature production technology and established safety data, making them the preferred platform for nucleic acid delivery. Prioritizing the advancement of LNP-mediated gene regulation therapies toward deeper preclinical development represents a path with potential for near-term progress.

Through multidisciplinary collaboration, the next generation of therapies based on nanotechnology holds promise for bringing substantive breakthroughs in improving the prognosis of newborns suffering from brain injury in the near future.

Abbreviations

HIE, hypoxic-ischemic encephalopathy; HI, hypoxic-ischemic; ROS, reactive oxygen species; DAMPs, damage-associated molecular patterns; RNS, reactive nitrogen species; BBB, blood-brain barrier; PAMAM, polyamidoamine; CP, cerebral palsy; LNPs, lipid nanoparticles; P-gp, P-glycoprotein; EVs, Extracellular vesicles; EPR, enhanced permeability and retention; PEG, polyethylene glycol; PLGA, poly (lactic-co-glycolic) acid; NF-κB, nuclear factor-kappa B; MAPK, p38 mitogen-activated protein kinase; D4-OH, generation-4 hydroxyl-functionalized PAMAM dendrimers; D-NAC, dendrimer-N-acetyl cysteine conjugate; G6-OH, generation-6 hydroxyl-terminated PAMAM dendrimers; SBE-β-CD, sulfobutyl ether β-cyclodextrin; FGR, fetal growth restriction; OGD, oxygen-glucose deprivation; PS-PEG, polystyrene-polyethylene glycol; AZ, azithromycin; MSCs, mesenchymal stromal cells; NSCs, neural stem cells; sEVs, small EVs; SOD, superoxide dismutase; BEV, brain rat tissue; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; VEGF, vascular endothelial growth factor; E4, estetrol; DCL, drug-in cyclodextrin in liposome; GFAP, glial fibrillary acidic protein; H_2S, hydrogen sulfide; Arhgap26, GTPase activating protein 26; Rgs1, Regulating G protein signaling 1; MRI, magnetic resonance imaging; CAT, catalase; GPx, glutathione peroxidase; Cur-Cu NPs, curcumin-copper complex nanoparticles; GSH, glutathione; HMONs, hollow manganese oxide nanoparticles; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labelling; PDA, polydopamine; $CeVO_4$, cerium vanadate; Nrf2, nuclear factor erythroid 2-related factor 2; GMP, Good Manufacturing Practice.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

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