Intranasal administration of stem cell-derived exosomes for central nervous system diseases

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From the Contents

Abstract

Exosomes, lipid bilayer-enclosed small cellular vesicles, are actively secreted by various cells and play crucial roles in intercellular communication. These nanosized vesicles transport internalized proteins, mRNA, miRNA, and other bioactive molecules. Recent findings have provided compelling evidence that exosomes derived from stem cells hold great promise as a therapeutic modality for central nervous system disorders. These exosomes exhibit multifaceted properties including anti-apoptotic, anti-inflammatory, neurogenic, and vasculogenic effects. Furthermore, exosomes offer several advantages over stem cell therapy, such as high preservation capacity, low immunogenicity, the ability to traverse the blood-brain barrier, and the potential for drug encapsulation. Consequently, researchers have turned their attention to exosomes as a novel therapeutic avenue. Nonetheless, akin to the limitations of stem cell treatment, the limited accumulation of exosomes in the injured brain poses a challenge to their clinical application. To overcome this hurdle, intranasal administration has emerged as a non-invasive and efficacious route for delivering drugs to the central nervous system. By exploiting the olfactory and trigeminal nerve axons, this approach enables the direct transport of therapeutics to the brain while bypassing the blood-brain barrier. Notably, exosomes, owing to their small size, can readily access the nerve pathways using this method. As a result, intranasal administration has gained increasing recognition as an optimal therapeutic strategy for exosome-based treatments. In this comprehensive review, we aim to provide an overview of both basic and clinical research studies investigating the intranasal administration of exosomes for the treatment of central nervous system diseases. Furthermore, we elucidate the underlying therapeutic mechanisms and offer insights into the prospect of this approach.

Key Words: central nervous system disease; exosome; extracellular vesicle; intranasal administration; stem cell

Introduction

Central nervous system (CNS) diseases pose a significant challenge. Stem cell therapies hold immense promise for ameliorating brain damage and restoring neural connectivity. Consequently, a multitude of basic and clinical investigations are currently underway (Kawabori et al., 2020; Yamazaki et al., 2020; Takamiya et al., 2023).

In recent years, considerable research efforts have been directed towards elucidating the role of exosomes as a crucial therapeutic mechanism of stem cells. Exosomes/extracellular vesicles, nano-sized vesicles ranging from 40-200 nm, are composed of a double lipid-layer membrane and harbor a plethora of molecules, including DNA, mRNA, microRNA (miRNA), and proteins (Kosaka et al., 2010). Stem cell-derived exosomes have exhibited remarkable potential in mitigating CNS diseases by virtue of their anti-apoptotic, anti-inflammatory, neurogenic, and angiogenic properties (Valadi et al., 2007; Tkach and Thery, 2016; Mathieu et al., 2019). Importantly, exosomes offer distinct advantages over the stem cells themselves, including the ability to undergo cryopreservation without degradation, reduced immunogenicity, and enhanced blood-brain barrier penetration (Ghasemi et al., 2023).

To maximize the therapeutic efficacy, the selection of an optimal transplantation route is of paramount importance in the field of cell-related therapy (Savitz et al., 2011). Among the commonly considered routes for exosome transplantation against CNS injuries, namely intravenous, intraarterial, and intracerebral routes, each method presents its limitations. Intravenous transplantation, for instance, results in systemic dilution, leading to a lower accumulation of exosomes in the brain. Intraarterial transplantation necessitates catheter insertion, which carries the potential risk of ischemic stroke. Intracerebral transplantation, on the other hand, may induce additional brain damage due to the use of transplantation needles. In light of these difficulties, intranasal transplantation is gaining recognition as a viable alternative route for exosome delivery in CNS diseases (Herman et al., 2021). The small size of exosomes enables their efficient absorption by the olfactory and trigeminal nerves, facilitating their axonal transfer to brain cells while bypassing the blood-brain barrier.

In this review, we summarize the intranasal administration of exosomes for CNS diseases, focusing on both basic and clinical research to clarify their efficacy and mechanism of action, as well as their limitations. This information aims to provide researchers with insights for developing future applications.

Search Strategy and Selection Criteria

A literature search was conducted on PubMed (https://www.ncbi.nlm.nih.gov/pubmed) to identify basic research articles on intranasal exosome administration for CNS diseases in May 2023. All years were chosen in the search. The search utilized the keywords "intranasal", "exosome or extracellular vesicle", and "brain". The articles included in the search were required to be written in English, relevant to CNS diseases, and specifically focused on stem cell-derived exosomes, while other articles were excluded. Additionally, we reviewed references cited within the selected papers from the preliminary search. The selection of articles and data collection were performed by one of the authors (SG). Ultimately, 41 articles were chosen that align with the objectives of this narrative review. The collected data encompassed various disease models, exosome sources, animal models, exosome dosage, treatment duration, labeling methods, and exosome engineering (**Figure 1**).

Overview of Intranasal Administration of Exosomes for Central Nervous System Diseases

Animal models and exosome dosage

Effects of intranasal exosome administration were investigated in models for various diseases (**Tables 1–7**). Animal models used for these experiments were solely mice and rats, and the feasibility of extrapolating these findings to larger animals would be necessary to adopt for clinical examination. The total doses of exosomes administrated via the intranasal route vary across the studies, ranging from 0.02 to 600 × 10^{10} particles, or 5 to 400 μg. The amounts of exosomes administered differ between the animals, with the protein weight of exosomes administered to mice tending to be smaller than that administered to rats. However, the particle numbers were mostly similar between the groups (**Figure 2**). Most articles described the amounts of exosomes either in terms of protein weight (μg) or the number of exosome particles, making it challenging to compare studies with different value representations. Three studies provided both the particle number and the

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volume of the exosomes. However, the protein weight of exosome per 1 × 10^{10} particles ranged between 5–200 μg, which may be attributed to different measurement methods. Standardization of exosome dosing is necessary to facilitate appropriate adoption in clinical use.

Source of exosomes

Twenty-four out of 31 exosomes (77.4%) were derived from humans, while the remaining 7 (22.6%) utilized exosomes from rodents. Mesenchymal stem cells (MSCs), including those derived from bone marrow, adipose tissue, and birth-associated sources, were commonly employed as cell sources for exosome extraction (**Figure 3**). MSCs offer several advantages over other cell sources, including lower costs for cell culturing and a high proliferation capacity. While both bone marrow-derived MSC and adipose-derived MSC can be used as autologous or allogenic sources, allogenic sources are potentially optimal due to their low immune profile of exosomes compared with the original MSC. Birth-associated tissue-derived MSCs also present a viable option as they can be obtained non-invasively from healthy volunteers and exhibit greater cell proliferation compared to other MSCs. Currently, there are no experiments specifically addressing the different sources of exosomes, and further comprehensive investigations are required to determine the most suitable cell source for intranasal exosome administration.

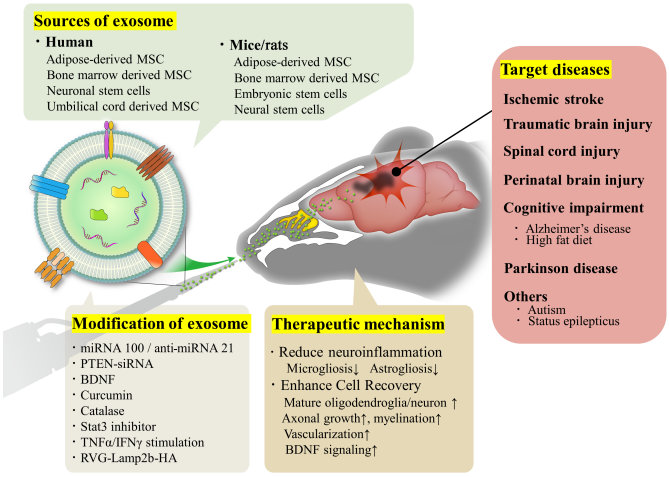

Figure 1 | **Illustrative description of the intranasal administration of exosome for CNS disease.**
The sources of the exosome are further categorized into human and mouse/rat origin. The modification methods of exosomes are listed. Two principle therapeutic mechanisms, reducing neuroinflammation, and enhancing cell recovery are delineated. Notably, the efficacy of intranasal exosome administration is demonstrated across various target diseases. Created with Adobe Illustrator. BDNF: Brain-derived neurotrophic factor; CNS: central neural system; IFNγ: interferon-gamma; miRNA: microRNA; MSC: mesenchymal stem cell; PTEN: phosphatase and tensin homolog; RVG: rabies virus glycoprotein; TNFα: tumor necrosis factor-α.

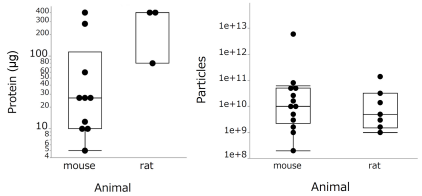

Figure 2 | **Different dosages of intranasal administration of exosome.**
Unpublished data.

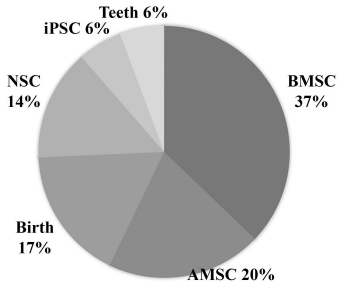

Figure 3 | **Different cell sources of exosome.**
AMSC: Adipose-derived MSC; BMSC: bone marrow-derived MSC; iPSC: induced pluripotent stem cell; MSC: mesenchymal stem cell; NSC: neural stem cell.

Exosome tracking

Twenty-four out of 34 articles (70.6%) have utilized exosomes labeling for in-vivo tracking. All of the studies demonstrate a significant presence of labeled exosomes not only in the olfactory nerve but also throughout the entire brain, including the brain stem and injured spinal cord.

The accumulation pattern of intranasally administered exosomes consistently indicates that these particles initially accumulate in the nasal cavity due to their mucoadhesive properties. Subsequently, they are gradually absorbed through the nasal mucosa, presumably entering perivascular spaces via branches of the olfactory and trigeminal nerves. Eventually, the exosomes migrate into the intracranial cavity and reach the site of injury. Fluorescence imaging performed after 24 hours of exosome administration clearly illustrates the progressive accumulation of these nanocarriers in the forebrains, a phenomenon that can persist for up to one to two days thereafter. Perets et al. (2019) showed distinctive patterns of exosomes accumulation in various brain diseases. Notably, in the stroke model, a significant concentration of exosomes was observed within the compromised striatum, whereas the olfactory bulb exhibited exosomes presence in the autism model. The authors reached the conclusion that exosome homing is driven by neuroinflammation. The biodistribution of intranasal exosome transplantation comparing with different methods has also been reported. Liang et al. (2020) and Zhai et al. (2022) showed that while intravenously transplanted exosomes mainly distributed to the liver, intranasally transplanted exosomes are mostly found in the brain. Tolomeo et al. (2023) conducted a comparative analysis of the intravenous, intratracheal, and intranasal administration routes for exosomes, revealing that intranasal administration predominantly yielded brain-specific exosome signals. In contrast, intravenous and intratracheal transplantation led to signals primarily emanating from the liver and lung. These data obtained consistently aligned across *in vivo*, *ex vivo*, and immunohistochemical analyses (Tolomeo et al., 2023).

Loading cargoes into exosomes

Exosomes act against target cells by transferring encapsulated components. Taking advantage of this property, several studies have utilized exosomes loaded with specific components, such as brain-derived neurotrophic factors (BDNF), miR-100, MALAT1, phosphatase and tensin homolog (PTEN), and curcumin (Kalani et al., 2016; Thomi et al., 2019a; Moss et al., 2021; Wang et al., 2021; Zhai et al., 2022). These cargoes can be easily loaded onto exosomes through genetic engineering of the original stem cells, resulting in enhanced efficacy through artificially loaded components. Modification of the exosome surface for better brain delivery has also been explored. Zhai et al. (2022) reported the brain-targeted modification of exosomes by tagging them with the rabies virus glycoprotein peptide, which specifically binds to the acetylcholine receptor expressed by neurons. These modified exosomes exhibited higher efficiency in delivering components compared to normal exosomes and showed better neurological recovery (Zhai et al., 2022).

Intranasal Exosome Administration for Central Nervous System Disease Models

Ischemic stroke

Ischemic stroke, typically caused by the occlusion of a brain blood vessel, leads to the deprivation of glucose and oxygen in its downstream branches, and thus emerging as the predominant cause of global disability. Presently, thrombectomy and recombinant tissue plasminogen activator therapy are standardly implemented. However, successful application of these treatments within the limited time window remains challenging, with only 5–10% of stroke patients reportedly eligible for such interventions, and approximately 50% of the patients will present neurological deficits even after successful recanalization (Henninger and Fisher, 2016). Stem cell therapy has shown efficacy across all three phases (acute, subacute, and chronic) in animal models (Kwak et al., 2018). However, due to the insufficient results of the clinical trials (Kawabori et al., 2020), other therapeutic modalities with a high accumulation of effective substances in the damaged area are strongly warranted. In that circumstance, stem cell-derived exosome administration is considered one good candidate and exploring intranasal administration of exosomes has just gained attention (Kalani et al., 2016; Rohden et al., 2021; Wang et al., 2023; Zhou et al., 2023; **Table 1**).

Kalani et al. (2016) conducted a study on the combined use of embryonic stem cell-derived exosomes and curcumin, a compound known for its radical scavenging and anti-inflammatory properties. The researchers found that this combination resulted in improved neurological recovery when administered intranasally. Although curcumin has previously shown therapeutic efficacy for brain damage, its low bioavailability has limited its effectiveness. However, the use of exosomes as carriers for curcumin addressed this limitation. Exosomes loaded with curcumin were found to be more stable, highly soluble, and concentrated in the body, thereby increasing its therapeutic potential. The study demonstrated that the curcumin-loaded exosomes led to a reduction in infarct volume, decreased brain edema, and improved neurological recovery. The underlying mechanisms for these positive outcomes were attributed to a decrease in reactive oxygen species production (Kalani et al., 2016).

Rohden et al. (2021) reported a study investigating the effects of intranasal transplantation of human adipose MSC-derived exosomes. They demonstrated significant improvements in neurological function in the group that received exosomes, and observed enhanced blood-brain barrier function to be the therapeutic mechanism. Zhou et al. (2023) demonstrated exosomes

Table 1 | **Intranasal administration of exosome against ischemic stroke model**

Source	Animal	Total dose of exosome	Timing	Labeling	Targeted content of EV	Effect	Reference
Mouse AMSC	Mice	10 μg	Day 1–3	PKH26	miR-760-3p	Reduce ferroptosis	Wang et al., 2023
Human iPSC derived MSC	Mice	5×10^{10} particles	2 hours	mCherry	BDNF	Reduce apoptosis Neurogenesis	Zhou et al., 2023
Human AMSC	Rats#1	80 μg	Day 1	PKH26	N/A	Better BBB integrity	Rohden et al., 2021
Mouse neural stem cell	Mice	N/A	Day 1–7	N/A	Curcumin	Reduce inflammation	Kalani et al., 2016

#1 Rats' weights were calculated as 400 g. AMSC: Adipose-derived mesenchymal stem cell; BBB: blood-brain barrier; EV: extracellular vesicles; iPSC: induced pluripotent stem cell; MSC: mesenchymal stem cell.

overloaded with BDNF exhibited enhanced neurogenesis, angiogenesis, synaptic plasticity, and fiber preservation, while also reducing inflammatory cytokines. They observed an upregulation of the BDNF/tropomyosin receptor kinase B signaling pathway in the ischemic brain, which is the result of the successful transfer of exosomal BDNF to the recipient cells (Zhou et al., 2023). Wang et al. (2023) further demonstrated that intranasally administered miR-760-3p enclosed in the adipose MSC-derived exosomes inhibited ferroptotic brain injury through the CHAC1 axis. This report highlights the significant association between miRNAs and their receptors in promoting cell survival following ischemic injury, thereby opening avenues for further investigation into the therapeutic mechanisms of miRNAs delivered through exosomes (Wang et al., 2023).

Traumatic brain injury and spinal cord injury

Traumatic brain injury and spinal cord injury are commonly caused by sudden mechanical damage to the brain or spinal cord. While advances in acute clinical care have led to improved survival rates (Carney et al., 2017), many patients still experience long-term disabilities, as evidenced by unchanged return-to-work rates (London, 1967; Cifu et al., 1997; Kawabori et al., 2022). Although, a recent study by Kawabori et al. (2021) reported the successful effect of intracerebral transplantation of bone marrow-derived MSC against chronic traumatic brain injury patients highlighting a potential treatment option, on-site cell processing procedures and highly invasive operative maneuver hamper worldwide application. In that circumstance, intranasal administration of MSC-derived exosomes has been intensively investigated as one optimal treatment option. Leon-Moreno et al. (2020) and Turovsky et al. (2022) demonstrated that intranasally administered exosomes obtained from birth-associated MSC successfully prevented neuronal apoptosis and achieved better neurological recovery (**Table 2**). Moss et al. (2021) demonstrated that MALAT1, a long noncoding RNA (lncRNA) highly expressed in MSC-derived exosomes, inhibited microglial activation through TrkC signaling. Guo et al. (2019) demonstrated the beneficial effects of intranasal administration of MSC-Exo loaded with phosphatase and tensin homolog small interfering RNA (ExoPTEN) in ameliorating the neurological deficits caused by complete spinal cord injury. The researchers observed that gold-labeled ExoPTEN could be detected near the site of spinal cord injury, promoting axonal growth and neovascularization while reducing microgliosis. Furthermore, spinal cord connectivity was confirmed through *in vivo* MRI imaging and electrophysiological studies.

Perinatal brain injury

Perinatal hypoxia-ischemic brain injury and preterm birth are the primary causes of morbidity and mortality for infants, which occur in 3 in 1000 live births. In addition to cerebral palsy, hypoxia-ischemic brain injury can result in long-term neurodevelopmental sequelae including behavioral, cognitive, and psychological problems as well as memory deficits. Oxygen deprivation leads to the activation of resident immune cells in the brain, which generate free radicals (Volpe et al., 2011). Targeting microglia-mediated inflammation is considered a potential therapeutic option for both hypoxic-ischemic brain injury and brain injury associated with preterm birth.

Their primary focus of intranasal administration of exosomes has been on the attenuation of neuroinflammation and the anti-apoptotic functions of exosomes (Sisa et al., 2019; Thomi et al., 2019a, b; Lawson et al., 2022; Turovsky et al., 2022; Labusek et al., 2023; Pathipati et al., 2023; **Table 3**). Thomi et al. (2019a, b) reported that rat pups induced with LPS and hypoxia demonstrated successful treatment through intranasal administration of exosomes derived from human umbilical cord mesenchymal stem cells (MSCs). The authors observed that the intranasally administered exosomes reached the frontal region of the brain within 30 minutes after administration and were distributed throughout the entire brain after 3 hours. Furthermore, the administered exosomes effectively reduced neuron-specific cell death, promoted normal myelination, and increased the counts of mature oligodendroglial and neuronal cells. In a subsequent study, Thomi et al. (2019a, b) revealed that exosomes interfered with Toll-like receptor 4 signaling in microglia, as they prevented the degradation of the nuclear factor κB inhibitor IκBα and the phosphorylation of molecules belonging to the mitogen-activated protein kinase family. This interference led to a reduction in neuroinflammation, resulting in the decrease of pro-inflammatory factors, including tumor necrosis factor-α, interleukin-6, and interleukin-1β (Thomi et al., 2019a, b). In line with these reports, Sisa et al. (2019) also demonstrated the successful attenuation of brain inflammation and cell apoptosis in the CA1 lesion of the hippocampus. Intranasal administration of exosomes derived from MSCs resulted in improved cognitive development for perinatal mice (Sisa et al., 2019).

Cognitive impairment

Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disease in humans and a major cause of dementia, posing a growing global health problem. The etiology and pathogenesis of AD are extremely complex, and amyloid plaque deposition and nerve fiber tangles are key pathological processes. Despite extensive efforts to develop small-molecule drugs and antibodies targeting amyloid beta or tau, these therapeutic approaches have consistently failed to improve cognitive function in patients. One such approach involves investigating hippocampal neural regeneration and controlling inflammation through microglial activation using exosomes derived from stem cells for AD (**Table 4**). Cone et al. (2021) demonstrated the reduction of glial activation and decreased accumulation of amyloid beta in the hippocampus through the intranasal administration of human bone marrow-derived MSCs. In a similar vein, Losurdo et al. (2020) showed the efficacy of intranasal injection of cytokine-preconditioned MSC-derived exosomes in AD model mice. Their findings revealed that exosomes suppressed microglial activation and reduced dendritic spine loss with only two intranasal administrations (Losurdo et al., 2020). Ma et al. (2020) conducted a study in which exosomes from human bone marrow-derived MSCs were found to accumulate in neurons and protect against amyloid beta oligomer-induced neuronal toxicity. Their proteomics analysis and RNA sequencing of extracellular vesicles revealed a significant upregulation of neuroprotective and neurogenesis-related substances, such as neprilysin and filamin-A, in the vesicles (Ma et al., 2020). These studies collectively support the notion that intranasal administration of exosomes possesses mechanisms of action beyond the mere removal of amyloid plaques from the brain. Consequently, this approach warrants significant attention in the treatment of AD.

High-fat diet and chronic hypertension-dependent memory impairment

Overnutrition and hypertension can disrupt normal cell signaling in the brain, potentially impacting synaptic function and adult neurogenesis, which in turn leads to cognitive impairment. Epidemiological evidence suggests that metabolic disorders, including insulin resistance and type 2 diabetes, contribute to accelerated brain aging and an increased risk of neurodegenerative diseases. In this context, intranasal administration of exosomes has emerged as a potential therapeutic strategy for memory impairment. The group of Fusco conducted a study showing that exosomes derived from neural stem cells have the ability to mitigate cognitive impairment induced by a high-fat diet (Spinelli et al., 2020; Natale et al., 2022). Natale et al. (2022) reported that intranasally administered exosomes inhibited the recruitment of transcription factors FoxO1 and FoxO3a resulting in neural stem cells within the hippocampus, leading to enhanced cell proliferation and reduced cell senescence. In a similar way, Spinelli et al. (2020) demonstrated that exosomes exerted an epigenetic effect by restoring the transcription of BDNF through modulation of cAMP response element-binding protein and tropomyosin receptor kinase B signaling pathways.

Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting over 1% of the population aged over 65 years, and nearly 5% of those aged over 80 years. The primary pathological feature of PD is the abnormal accumulation of α-synuclein (α-syn) in dopaminergic neurons in the substantia nigra of the midbrain. However, no effective treatment has been developed to halt the progression of the disease thus far. One of the pathophysiological mechanisms implicated in PD is the abnormal activation of microglia, which leads to the release of pro-inflammatory factors. Consequently, targeting this process has emerged as a therapeutic strategy for exosome-based interventions. The successful improvement in motor symptoms and cognitive functions of a PD animal model through the administration of exosomes derived from human teeth mesenchymal stem cells (**Table 5**). The normalization of tyrosine hydroxylase expression in the substantia nigra was identified as one potential therapeutic mechanism (Narbute et al., 2019, 2021). This substance was loaded with curcumin, an agent known to inhibit α-syn aggregation, enhance autophagy for the clearance of existing aggregates, and reduce the toxicity of aggregates to dopaminergic neurons. The substance's outer layer was embedded with rabies virus glycoprotein (RVG29) peptides, facilitating targeted delivery through the recognition of acetylcholine receptors. The engineered exosomes successfully traversed multiple membrane barriers, actively migrated to the lesion site, and enabled efficient drug enrichment in damaged dopaminergic neurons. By directly releasing drugs through membrane fusion, curcumin effectively removed α-syn aggregates, reducing their neurotoxicity. Additionally, the endogenous miR-133b present in the exosomes promoted neuronal axon growth and functional recovery, leading to significant

Table 2 | **Intranasal administration of exosome against traumatic brain injury and spinal cord injury model**

Disease	Source	Animal	Total dose of exosome	Timing	Labeling	Targeted content of EV	Effect	Reference
TBI	Human BMSC	Mice	2.5×10^{10} particles	1.5 hours	PKH26	N/A	Reducing microglial activation	Kodali et al., 2019
TBI	Human PMSC	Rats	1.4×10^{10} particles	1, 4, 7 days	N/A	N/A	Anti-apoptosis through Ca influx	Turovsky et al., 2022
TBI	Human AMSC	Mice	10 μg	2 days	mCherry/GFP	MALT1	Reduce microglial inflammation	Moss et al., 2021
TBI	Human EMSC	Mice#1	12.5 μg	1 day	N/A	N/A	Anti-apoptosis	Leon-Moreno et al., 2020
SCI	Human BMSC	Rats	0.1×10^{10} particles	2 hours	Gold nanoparticle/ PKH26	PTEN	Anti-inflammation, axonal regeneration	Guo et al., 2019

#1 Weights of the mice were set as 25 g. AMSC: Adipose-derived mesenchymal stem cell;BMSC: bone marrow-derived mesenchymal stem cell; EMSC: endometrial mesenchymal stem cell; EV: extracellular vesicle; PMSC: placenta-derived mesenchymal stem cell; SCI: spinal cord injury; TBI: traumatic brain injury.

Table 3 | **Intranasal administration of exosome against perinatal ischemic injury model**

Source	Animal	Total dose of exosome	Timing	Labeling	Targeted content of EV	Effect	Reference
Human MSC	Mice	5×10^6 cells equivalents	1, 3, 5 days	N/A	N/A	Anti-inflammation/ Neurogenesis	Labusek et al., 2023
Human PMSC	Rat	3.2×10^{10} particles	1–10 days	N/A	N/A	Anti-apoptosis through Ca influx	Turovsky et al., 2022
Mouse NSC	Mice	1.6×10^{10} particles	30 min & 1 day	PKH67	N/A	Anti-apoptosis	Lawson et al., 2022
Mouse BMSC	Mice	5 μg	0 min	CellVue Claret Far Red Fluorescent Cell Linker kit	N/A	Anti-inflammation (microglia/ macrophage)	Pathipati et al., 2023
Human UC-MSC	Rat #2	400 μg	Before ischemia	IRDye	N/A	Anti-apoptosis/myelination	Thomi et al., 2019a
Human BMSC	Mice	0.1×10^{10} particles	0 min	N/A	N/A	Anti-apoptosis/inflammation	Sisa et al., 2019
Human UC-MSC	Rat #2	400 μg	Before ischemia	PKH26	N/A	Anti-microglial inflammation	Thomi et al., 2019b

Weight of 9–15 days old C57Bl/6 mouse was set to 10 g. #2 weight of pup day 2 rat was set to 8 g. BMSC: Bone marrow-derived mesenchymal stem cell; EV: extracellular vesicle; min: minutes; MSC: mesenchymal stem cell; NSC: neural stem cell; PMSC: placenta-derived mesenchymal stem cell; UC: umbilical cord.

Table 4 | **Intranasal administration of exosome against cognitive impairment model**

Disease	Source	Animal	Total dose of exosome	Course of treatment	Labeling	Targeted content of EV	Effect	Reference
Alzheimer's disease	Human BMSC	Mice	6.2×10^{10} particles	Every 4 days for 4 months	N/A	N/A	Reduce microglial inflammation, amyloid beta	Cone et al., 2021
Alzheimer's disease	Human AMSC	Mice#1	280 μg	Every 2 days for 2 weeks	PKH26/125I	N/A	Anti-inflammation and rescue memory deficits	Ma et al., 2020
Alzheimer's disease	Human BMSC	Mice	60 μg	N/A	PKH26	N/A	Anti-inflammation and increase dendritic spine density	Losurdo et al., 2020
Chronic hypertension	Human AMSC	Rat	14×10^{10} particles	1–6 week (weekly)	PKH26	N/A	Improve cognitive function	Guy et al., 2022
High-fat diet	Mice NSC	Mice	27 μg	3 times/week for 6 weeks	Exo-Glo	N/A	Reduce senescence in hippocampus cells	Natale et al., 2022
High-fat diet	Mice NSC	Mice	27 μg	3 times/week for 6 weeks	ExoGlowTM	Anti-CREB	Improve cognitive function	Splinelli et al., 2020

#1 9-month-old mice's weights were set as 40 g. AMSC: Adipose-derived mesenchymal stem cell; BMSC: bone marrow-derived mesenchymal stem cell; NSC: neural stem cell.

Table 5 | **Intranasal administration of exosome against Parkinson's disease model**

Source	Animal	Total dose of exosome	Course of treatment	Labeling	Targeted content of EV	Effect	Reference
Human teeth MSC	Rats	0.5×10^{10} particles	17 consecutive days	N/A	N/A		Narbute et al., 2021
Human teeth MSC	Rats	0.3×10^{10} particles	15 consecutive days	N/A	N/A	Reduce neuronal cell loss	Narbute et al., 2019
Mouse BMSC	Mice	0.5×10^{10} particles	15 consecutive days	SPIO	miR-133b curcumin	Anti-inflammation anti α-syn aggregation	Peng et al., 2022

α-syn: α-Synuclein; BMSC; bone marrow-derived mesenchymal stem cell; MSC: mesenchymal stem cell; SPIO: superparamagnetic iron oxide nanoparticles.

alleviation of neuroinflammation (Peng et al., 2022). In summary, the studies demonstrate promising therapeutic potential for intranasal exosome interventions in PD, and these findings contribute to our understanding of the disease's pathophysiology and provide valuable insights for the development of effective treatments.

Other neurological diseases

Autism

Autism, also known as autistic spectrum disorder, is a type of neurodevelopmental disorder characterized by social interaction deficits, cognitive inflexibility, and communication disorder, which typically emerge before the age of 3 years. Despite the increasing prevalence of autism, effective prevention and treatment options remain limited. The etiology of autism is highly complex, involving both genetic and epigenetic factors (Muhle et al., 2004; Geschwind, 2011; Eshraghi et al., 2018). Due to the unclear pathological mechanism and the diverse nature of the disease, currently approved pharmacological treatments primarily target comorbid behaviors commonly observed in autism, such as anxiety, hyperactivity, and impulsive-related behaviors. The intranasal administration of exosomes has been investigated to modify neuroinflammation and promote neural circuit regeneration in animal models of autism (**Table 6**; Perets et al., 2018, 2020; Liang et al., 2020). Perets et al. (2018, 2020) demonstrated that animals receiving exosomes exhibited improved male-to-male social interaction and reduced repetitive behaviors. Furthermore, they observed an increase in male-to-female ultrasonic vocalizations and a significant improvement in maternal behaviors. The authors also reported higher expression levels of GABA receptors and oxytocin in animals that received exosome treatment.

Status epilepticus

Status epilepticus (SE), a medical emergency that is typically terminated through antiepileptic drug treatment, leads to chronic hippocampus dysfunction. This dysfunction is characterized by persistent inflammation with activation of microglia and monocyte infiltration, loss of inhibitory interneurons, aberrant and waned neurogenesis, and hippocampus-dependent

cognitive and memory impairments (Vezzani et al., 2015; Trinka and Kalviainen, 2017). Intranasal exosome administration has been extensively studied as a potential intervention to ameliorate the neurological deficit after SE. Kodali et al. (2019) investigated the effect of intranasally administered exosomes derived from human MSC on neurons and microglia in a rat model of kainite-induced SE injury. The authors injected PKH26-labeled extracellular vesicles into rats that underwent 2 hours of SE. Six hours later, a higher percentage of neurons incorporated the extracellular vesicles in the hippocampal CA1 subfield and the entorhinal cortex, which are regions that typically display neurodegeneration after SE in animals (Kodali et al., 2019). Long et al. (2017) demonstrated that intranasal administration of exosomes secreted from human bone marrow-derived MSC reached the hippocampus within 6 hours of administration. This administration reduced the loss of glutamatergic and GABAergic neurons, diminished inflammation in the hippocampus, and resulted in long-term cognitive and memory function improvement (Long et al., 2017). In another study, Upadhya et al. (2020) investigated the effects of intranasal transplantation of exosomes derived from neuronal stem cells obtained from induced pluripotent stem cells. This method allows for the acquisition of neural stem cells without the need for cell harvesting from volunteers or donors and offers the possibility of ultimate cell expansion and exosome retraction. The researchers found that intranasal administration of exosomes enhanced neurogenesis in the hippocampus following SE (Upadhya et al., 2020). Taken together, these findings indicate that intranasal administration of exosomes has the potential to restore the neural circuitry of the hippocampus and improve cognitive function following SE.

Comparison of individual diseases

In the context of distinct diseases, a comparative analysis reveals noteworthy variations. Acute conditions such as stroke, head trauma, spinal cord injury, perinatal brain injury, and epilepsy frequently necessitated intranasal exosome administration lasting only a few days. In contrast, chronic ailments like AD, autism, and ALS often demanded intranasal exosome administration over the span of one week or more. These disparities stem from two primary factors: exosome deactivation and treatment duration. While exosomes derived from stem cells exhibit extended *ex-vivo* preservation, the precise half-life of intranasally administered exosomes remains undisclosed. This knowledge gap is partially informed by studies involving intravenous exosome administration, indicating a half-life of a few minutes (Yamashita et al., 2016). This limited persistence suffices to mitigate acute inflammatory and apoptotic processes; however, addressing chronic conditions frequently mandates multiple administrations. Determining the optimal duration for intranasal exosome administration specific to each ailment stands as a pivotal consideration.

Intranasal Exosome Administration in Clinical Trials

We conducted a survey of clinical studies on intranasal administration of exosomes using "Clinicaltrial.gov" and identified three relevant studies (**Table 7**).

Refractory focal epilepsy

An exploratory Phase I clinical study on induced pluripotent stem cell-derived exosomes (GD-iEXo-002) as nasal drops for the treatment of refractory focal epilepsy is being conducted at Peking Union Medical College Hospital in China, sponsored by Guidon Pharmaceutics Ltd. The study includes four treatment groups: a low-dose group (2 μg), medium-dose groups (6 μg and 18 μg), and a high-dose group with an unknown dosage in 200 μL. Exosomes are administered via nasal drip for 12 weeks. The primary outcome measure is safety monitoring, and secondary outcome measures include seizure frequency, scalp electroencephalogram monitoring, and MRI neuroimaging. The study started in June 2023, with a planned completion date of November 2025. No results have been posted to date.

AD

An evaluation of the safety and efficacy of exosomes derived from allogenic adipose MSCs (MSCs-Exos) in subjects with AD is being conducted at Ruijin Hospital Affiliated to Shanghai Jiao Tong University in Shanghai, China. The study, sponsored by Cellular Biomedicine Group Ltd., is an open-label, single-center Phase I/II trial. Three different dosages of exosomes (low: 5 μg, medium: 10 μg, high: 20 μg) are administered twice a week for 12 weeks via nasal drops (total volume: 1 mL). Safety monitoring is the primary outcome measure, and cognitive functional tests, MRI neuroimaging, and positron emission tomography are planned as secondary endpoints to assess efficacy. The study started in July 2020, with a planned completion date of August 2022. However, no results have been posted yet.

Extremely low birth weight infants

A blinded randomized controlled trial is planned to investigate the intranasal administration of MSC-derived exosomes in extremely low birth weight infants with a gestational age of 25/0–27/6 weeks. The study will take place in Moscow, Russian Federation. In the exosome treatment group, infants will receive exosomes obtained from a daily conditioned culture medium of 120 million MSCs, and the control group will not receive exosomes. The exosomes will be suspended in 500 μL of phosphate buffer and administered

Table 6 | **Intranasal administration of exosome against psychiatric and other neurological disease model**

Disease	Source	Animal	Total dose of exosome	Course of treatment	Labeling	Targeted content of EV	Effect	Reference
Alcoholism	Human AMSC	Rats	0.15×10^{10} particles (=7.5 μg)	Once a week for 5 weeks	PKH26	N/A	Reduce brain oxidative stress Less alcohol consumption	Ezquer et al., 2019
ALS	Mouse AMSC	Mice	26 μg	Every 4 days	USPIO	N/A	Preserved motor neuron	Bonafede et al., 2020
Autism	Human UC-MSC	Mice	400 μg	Weekly for 4 weeks	Dir/Dio	N/A	Smaller inflammation and cognitive recovery	Liang et al., 2020
Autism	Human BMSC	Mice	0.02×10^{10} particles	4 times for 8 days	PKH26	N/A	Increased GABA receptors and better function	Perets et al., 2020
Autism	Human BMSC	Mice	1×10^{10} particles	6 times for 12 days	PKH26	N/A	Better social function	Perets et al., 2018
Glioblastoma	Neural stem cell	Mice	600×10^{10} particles	Every other day	ICG-NIR Dy	CXCR4 miR-100 anti-miRNA-21	Better survival period	Wang et al., 2021
Schizophrenia	Human MSC	Mice	0.3×10^{10} particles	14 consective days	PKH26	N/A	Preserve GABAergic interneuron Reduce serum glutamate level	Tsivion-Visbord et al., 2020
Status epilepticus	Human iPSC-derived NSC	Mice	5×10^{10} particles (=25 μg)	2 hours after symptom	PKH26	N/A	Anti-inflammation	Upadhya et al., 2020
Status epilepticus	Human BMSC	Mice	1×10^{10} particles	Soon after epilepticus ceasing	PKH26	N/A	Exosomes are well observed in the damaged neurons	Kodali et al., 2019
Status epilepticus	Human-BMSC	Mice	0.15×10^{10} particles (=30 μg)	2 hours and 29 hours after symptom	PKH26	N/A	Anti-inflammation Less neuronal loss"	Long et al., 2017

ALS: Amyotrophic lateral sclerosis; AMSC: adipose-derived mesenchymal stem cell; BMSC: bone marrow-derived mesenchymal stem cell; iPSC: induced pluripotent stem cell; NSC: neuronal stem cell; UC-MSC: umbilical cord mesenchymal stem cell; USPIO: ultra-small superparamagnetic iron oxide nanoparticles.

Table 7 | **List of clinical trials adopting intranasal administration of exosome for neurological disease**

Disease	NCT number	Country	Phase	Exosome origin	Dose	Duration	Primary outcome	Secondary outcome
Epilepsy	05886205	China	1	iPSC	2–18 μg/200 μL	12 weeks	Safety	Seizure frequency
Alzheimer's disease	04388982	China	1/2	Adipose MSC	5–20 μg/1 mL	Twice a week for 12 weeks	Safety	Cognitive function Aβ in serum and CSF
Preterm birth	05490173	Russia	N/A (Randomized)	MSC	Culture medium of 120 million MSC/500 μL	Days 1, 3, 5, 7, 9	Safety	Mental development

Aβ: Amyloid-beta; CSF: cerebrospinal fluid; iPSC: induced pluripotent stem cell; MSC: mesenchymal stem cell.

in each nostril at a volume of 50 μL. The therapeutic course will consist of 5 instillations with a 1-day interval between each administration. The primary outcome measure of the study includes assessing the incidence of death and survival with severe intraventricular hemorrhage or cystic periventricular leukomalacia. Biomarkers of perinatal brain injury, such as S-100, NSE, EPO, and mRNA, will also be tested. The study is estimated to start in October 2022, with a planned enrollment of 10 infants. However, recruitment has not yet been initiated at this time.

Future Perspectives

Intranasal administration of exosomes derived from stem cells holds promise for improving neurological diseases, given its non-invasive nature and high brain infiltration capacity. However, several unresolved issues need to be addressed before its full clinical application. Firstly, there is a need to establish efficient separation technologies to obtain an adequate amount of exosomes from the culture medium. Currently, ultracentrifugation is the most common method, but it only allows for the collection of small quantities of exosomes at a time. Secondly, a quality control standard for clinical-grade exosomes must be established, as the definition of exosomes remains unclear. Thirdly, the optimal dosage of exosomes for human patients is still unknown. Developing effective tracking methods for monitoring exosome biodistribution would be essential. Lastly, the potential adverse effects of exosomes in patients are not yet fully understood. Therefore, extensive basic researches, as well as long-term and large-scale clinical trials, are necessary to facilitate the translation of exosome therapy into clinical practice.

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